

Association of the Plasminogen Activator Inhibitor-1 Gene 4G/5G Polymorphism With ST Elevation Acute Myocardial Infarction in Young Patients

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Introduction and objectives. To investigate the role of the 4G/5G polymorphism in the plasminogen activator inhibitor-1 (PAI-1) gene in patients with ST elevation myocardial infarction (STEMI) aged ≤ 45 years and its influence on regulation of the plasma PAI-1 concentration.

Methods. This case-control study included 127 consecutive patients aged ≤ 45 years with a diagnosis of STEMI who were admitted to a cardiovascular intensive care unit and 127 controls recruited between January 2006 and March 2007. Participants were genotyped for the 4G/5G polymorphism using the polymerase chain reaction and restriction fragment length polymorphism analysis, and their plasma PAI-1 concentrations were measured. Informed consent was obtained from all participants.

Results. There was a significant difference in genotype distribution between the 2 groups ($P < .002$). The 4G allele occurred more frequently in the patient group ($P = .032$). In addition, there were significant independent associations between STEMI and the 4G allele (ie, 4G/4G plus 4G/5G; odds ratio [OR] = 2.29; 95% confidence interval [CI], 1.12-4.68; $P = .022$), smoking (OR = 23.23; 95% CI, 8.92-60.47; $P < .001$), a family history of cardiovascular disease (OR = 4.66; 95% CI, 2.06-10.52; $P = .001$) and hypertension (OR = 5.42; 95% CI, 1.67-17.56; $P = .005$). The plasma PAI-1 concentration was higher in individuals who were homozygous for the 4G allele ($P < .001$).

Conclusions. The study findings indicate that the 4G allele is an independent risk factor for acute myocardial infarction in young patients, as are smoking, hypertension, and a family history of inherited cardiovascular disease.

Key words: Fibrinolysis. Myocardial infarction. Thrombolysis. Plasminogen activator inhibitor-1. Coagulation.

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Received April 11, 2008.

Accepted for publication December 11, 2008.

Asociación entre el polimorfismo 4G/5G en el gen del inhibidor del activador del plasminógeno-1 (PAI-1) y el infarto agudo de miocardio con elevación del ST en pacientes jóvenes

Introducción y objetivos. Determinar la participación del polimorfismo 4G/5G en el gen del inhibidor del activador del plasminógeno tipo 1 (PAI-1) en pacientes con infarto agudo de miocardio con elevación del segmento ST y edad ≤ 45 años y su influencia en la regulación de la concentración plasmática de PAI-1.

Métodos. En un estudio de casos y controles se incluyó, entre enero de 2006 y marzo de 2007, a 127 pacientes consecutivos con diagnóstico de infarto agudo de miocardio con elevación del segmento ST ingresados a la unidad de cuidados intensivos cardiovasculares y 127 controles. Se realizó genotipificación del polimorfismo 4G/5G mediante técnica de reacción en cadena de la polimerasa-polimorfismos en la longitud del fragmento de restricción, y la determinación de la concentración plasmática de PAI-1. Todos los pacientes firmaron consentimiento informado.

Resultados. Se identificó una diferencia con significación estadística en la distribución genotípica entre los grupos ($p < 0,002$). La frecuencia del alelo 4G fue mayor en el grupo de estudio ($p = 0,032$). Se asociaron en forma independiente al infarto agudo de miocardio con elevación del segmento ST el alelo 4G (4G/4G + 4G/5G) (odds ratio [OR] = 2,29; intervalo de confianza [IC] del 95%, 1,12-4,68; $p = 0,022$), el tabaquismo (OR = 23,23; IC del 95%, 8,92-60,47; $p < 0,001$), el antecedente familiar de enfermedad cardiovascular (OR = 4,66; IC del 95%, 2,06-10,52; $p < 0,001$) y la hipertensión arterial (OR = 5,42; IC del 95%, 1,67-17,56; $p = 0,005$). Las concentraciones plasmáticas de PAI-1 fueron mayores en los homocigotos 4G ($p < 0,001$).

Conclusiones. Estos resultados indican que el alelo 4G es un factor independiente de riesgo de infarto agudo de miocardio en pacientes jóvenes, al igual que el tabaquismo, la hipertensión arterial y los antecedentes hereditarios familiares de enfermedad cardiovascular.

Palabras clave: Fibrinólisis. Infarto de miocardio. Trombosis. PAI-1. Coagulación.

ABBREVIATIONS

PAI-1: plasminogen activator inhibitor type 1
STEMI: ST elevation myocardial infarction

INTRODUCTION

Cardiovascular disease is the most important cause of morbidity and mortality throughout the world, and ST elevation myocardial infarction (STEMI) the most common cause of death in Mexico.¹ About 9% of new events occur in subjects under 45 years of age; it is estimated that a genetic element is involved in some 20%-60% of these cases.²

Certainly, it is known that fibrinolytic activity is reduced in patients under 45 who suffer an acute myocardial infarction (AMI).³ Plasminogen activator inhibitor type-1 (PAI-1) is the main physiological inhibitor of the activity of the fibrinolytic system. It achieves this via the inhibition of tissue plasminogen activator (tPA) and via the inhibition of the inhibitor of the urokinase type activator (uPA). An increase in plasma concentration is therefore associated with thrombotic events.⁴ The polymorphism consisting of a single insertion/deletion of a guanine base at position 675 in the promoter region of PAI-1 gives rise to 2 alleles—4G and 5G—which differ in their regulation of the concentration of PAI-1.^{5,6} Individuals who are homozygous for the 4G allele (4G/4G) have higher concentrations of PAI-1 than those who are homozygous for 5G (5G/5G). Individuals who are heterozygous (4G/5G) have intermediate levels.⁷ The 5G allele possesses an additional repressor protein binding site that is absent in the 4G allele; as a consequence the 4G allele produces up to six times more mRNA and is associated with higher PAI-1 activity.

The data available regarding the risk of 4G/5G polymorphism in the development of AMI are contradictory. Some studies have shown an association between such polymorphism and an increased risk for STEMI,⁸⁻¹² while others suggest the risk to be reduced¹³ or find no association at all.¹⁴⁻¹⁶ An increase in the plasma concentration of PAI-1 is, however, associated with higher mortality¹⁷ and the suffering of a second AMI in patients under 45 years of age.¹⁸

Different populations around the world show variation in terms of the allelic frequencies of 4G and 5G, with the 4G allele seen more commonly in Asian (59%),¹⁹ Caucasian (51%),²⁰ Spanish (47%),²¹ and Indian (54%)²² populations than in Mexican or Afroamerican (34%)²³ populations (25%).²⁴ This leads to variations in the plasma concentration of PAI-1 between populations, as well as in its interaction with other regulating factors such as triglycerides, glucose, insulin, hypertension, and smoking.^{25,26} This contributes to differences in susceptibility to the development of cardiovascular disease among different populations, which is more common among American Indians (15%-20%) and Caucasians (20%-25%) than among Africans (1%-5%).²⁷

The aim of the present work was to assess the relationship between carriage of the 4G or 5G alleles, PAI-1 production, and the development of STEMI in Mexican patients aged 45 years or younger.

METHODS

The STEMI subjects in this study were consecutive patients admitted to the intensive care unit of the Hospital de Cardiología del Centro Médico Nacional Siglo XXI over the period January 2006-March 2007. Patients (cases) were paired with controls according to age and sex.

The criteria for STEMI diagnosis (ESC/AHA/ACC)^{28,29} were chest pain lasting 20 min or more, the elevation of MB creatine kinase (CK-MB) or troponins to the 99th percentile or higher, and elevation of the ST segment by 2 mm in 2 contiguous leads in V₁₋₃ and by 1 mm in the remaining leads.

All patients gave their signed, informed consent to be included. The study protocol was approved by the Ethics and Research Committee of the Instituto Mexicano del Seguro Social according to the principles outlined in the Declaration of Helsinki.

The presumably healthy control subjects were blood donors who attended the same hospital. By Mexican Law (NOM-003-SSA2-1993), blood (and its components) for therapeutic use must be provided by donors who have no risk factors. According to the European Society of Cardiology, risk factors for both patients and blood donors were deemed to be:

- Diabetes mellitus: subjects with a fasting blood sugar level of >126 mg/dL or undergoing blood sugar-lowering treatment
- Dyslipidemia: subjects with a cholesterol concentration of >200 mg/dL or undergoing treatment for this condition

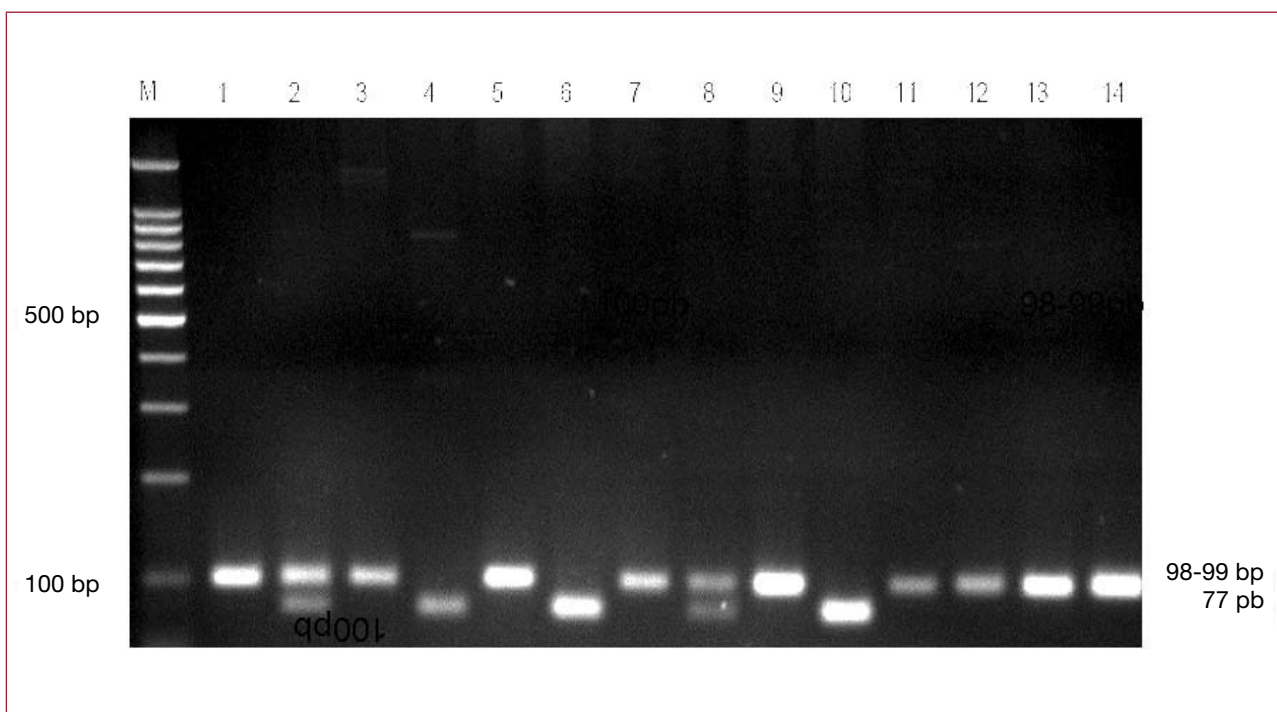


Figure 1. Figure. Agarose gel electrophoretic analysis of the 4G/5G polymorphic region of PAI-1. M represent 100 bp molecular weight marker; lines 1, 3, 5, 7, 9, 11, and 13 represent the 98-99 bp polymorphic fragment; lines 4, 6, and 10 represents the fragments corresponding to the genotype 5G/5G after digestion with Bsl I (77, 22 bp); lines 2 and 8 represent the fragments corresponding to the genotype 4G/5G after digestion (98, 77, 22 bp); lines 12 and 14 represent the fragments corresponding to the genotype 4G/4G after digestion (98 bp)

– High blood pressure: subjects with a threshold systolic blood pressure of 140 mm Hg and diastolic pressure of 90 mm Hg, or undergoing medical treatment for this condition

All patients with known thrombophilic disease, valve disease and congenital heart disease were excluded.

Determination of Plasma PAI-1

Blood samples were extracted with minimum stasis 6 weeks after STEMI in tubes containing citrate as an anti-coagulant. All samples were collected between 8:00 and 9:00 h to avoid variations due to the circadian rhythm. Samples were centrifuged at 3000 g for 25 min at 4°C to avoid the contamination of the plasma with platelets. They were then stored in aliquots of 0.5 mL at –70°C until use. The plasma concentration of PAI-1 was determined immunoenzymatically by ELISA (Coaliza PAI-1, Chromogenix, Milan, Italy).

Determination of Genotypes

Genomic DNA was obtained from peripheral blood (leukocyte concentrate) using the commercial

QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Genotyping of the 4G/5G polymorphism in the PAI-1 promoter region was performed by PCR using the following oligonucleotides: 5'-CACAGAGAGAGTCTGGCCACGT-3' (sense) and 5' CCAACAGAGGACTCTTGGTCT-3' (antisense).³⁰ Reactions were performed in volumes of 50 µL with 0.06 mol of each oligonucleotide, 1 U of Taq DNA polymerase, 1.5 mmol of MgCl₂ and 0.1 mmol of each dNTP. All reagents not supplied in the above kit were obtained from Promega (Madison, Wisconsin, USA). The reaction conditions were as follows: initial denaturation at 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 30 s, alignment at 60°C for 30 s, and an extension step at 72°C for 30 s, followed by a final linear extension step at 72°C for 1 min. Amplification products of 99 bp (5G) and 98 bp (4G) were obtained. Products (25 µL) were subjected to digestion with 1 U of the specific restriction enzyme Bsl I (New England Biolabs, Beverly, Massachusetts, USA) at 55°C. The DNA fragments were separated by electrophoresis in 4% agarose gels (BIO-RAD Laboratories, Hercules, California, USA) and visualized using ethidium bromide (Figure). All samples were processed in duplicate. Some were subject to sequencing.

TABLE 1. Clinical and Demographic Data of the Patients and Controls

Variable	Patients (n=127)	Controls (n=127)	P
Age, mean (SD), y	40 (4.6)	40 (4.1)	.53 ^a
Men, %	83.3	82.6	NS
BMI	28.1 (3.4)	27.1 (3.9)	.50 ^a
Smokers, %	65.8	13.3	<.001 ^b
High blood pressure, %	43.6	9.4	<.001 ^b
Diabetes mellitus, %	36	7.8	<.001 ^b
Dyslipidemia, %	47.6	8.6	<.001 ^b
Family background of coronary heart disease, %	42.5	11.8	<.001 ^b
Location of AMI, %			
Anterior wall	38.6		
Lower wall	59.8		
Posterolateral	1.6		
Type of infarction, %			
With elevated ST segment	100		
History of angina	Absent		
Creatinine	0.91 (0.37)	0.83 (0.27)	.13 ^a

AMI indicates acute myocardial infarction; BMI, body mass index.

^aStudent *t* test.

^b χ^2 test.

Values are means (SD) unless otherwise indicated.

Statistical Analysis

Continuous variables were expressed as means (standard deviation) (SD). Categorical variables were expressed as percentages. Differences between continuous variables were analyzed using the Student *t* test, while those between categorical variables were analyzed using the χ^2 test. Differences in mean plasma PAI-1 concentrations between genotypes were analyzed by ANOVA. Odds ratios (OR) and 95% confidence intervals (95% CI) associated with conventional risk factors and 4G/5G polymorphism were determined by logistic regression. A *P* value less than .05 was considered significant. All calculations were performed using SPSS (Statistical Package for the Social Sciences) software v.13 (SPSS Inc, Chicago, Illinois, USA) and EpiInfo software v. 3.3.2. 2005.

RESULTS

The study population was composed of 127 patients, all ≤ 45 years of age, with a diagnosis of STEMI, and 127 age- and sex-matched controls. The prevalence of risk factors was high among the patient group; those associated with STEMI were smoking (OR=12.64; 95% CI, 6.46-25), high blood pressure (OR=5.63; 95% CI, 2.68-12.01), diabetes mellitus (OR=5.58; 95% CI, 2.52-12.63), dyslipidemia (OR=5.79; 95% CI, 2.69-12.68), and a family background of cardiovascular disease (OR=5.52; 95% CI, 2.78-11.09). Table 1 shows the distribution of the localization of AMI.

Among the patients, the most common genotype was 4G/5G (50.4%), followed by 5G/5G (42.5%), and finally 4G/4G (7.1%). The allelic frequency of 4G among the patients was 32.3%; that of 5G was 67.7%. In the control group, 13.4% of the subjects had the 4G/4G genotype, 30% had the 4G/5G genotype, and 56.6% had the 5G/5G genotype. The allelic frequency of 5G was 71.6% in this group. The difference in genotype distribution between the 2 groups was significant ($P < .002$), but no significant difference was seen in the allelic frequencies between the patient and control groups ($P = .46$). Univariate analysis identified a risk of STEMI for those carrying the 4G allele (ie, those with the genotypes 4G/4G and 4G/5G) compared to those with the 5G/5G genotype (OR=1.77; 95% CI, 1.04-3) (Table 2). No significant differences were seen between those carrying either type of allele in terms of the mean left ventricular ejection fraction ($P = .58$). The 5G/5G homozygotes had a mean creatine kinase concentration of 956 (767) U/L, while that of carriers of the 4G allele was 1756 (1661) U/L ($P = .01$). They also had a mean troponin I (TnI) concentration of 8.5 (8.4) ng/dL, while that of carriers of the 4G allele was 15.6 (13.08) ng/dL ($P = .05$). With respect to inflammatory status, the concentration of fibrinogen was higher among carriers of the 4G allele (585 [187] mg/dL) compared to the 5G/5G homozygotes (471 [133] mg/dL) ($P = .02$). The leukocyte count of the 4G carriers was 10 754 (2232)/ μ L and 10 436 (3113)/ μ L ($P = .64$) for the 5G homozygotes. Percutaneous transluminal coronary angioplasty (PTCA) was performed in 38 patients, 24 of whom were 5G

TABLE 2. Distribution of the 4G/5G Genotypes and Allelic Frequencies

	Patients (n=127)	Controls (n=127)	P ^a
Genotype, n (%)			
4G/4G+4G/5G	73 (57.48)	55 (43.3)	.032
5G/5G	54 (42.52)	72 (56.7)	
4G/4G	9 (7.1)	17 (13.4)	.024
4G/5G	64 (50.4)	38 (30)	
5G/5G	54 (42.5)	72 (56.6)	
Allelic frequency, n (%)			.461
4G	82 (32.3)	72 (28.4)	
5G	172 (67.7)	182 (71.6)	

PAI-1 indicates plasminogen activator inhibitor-1.

^a χ^2 test.

homozygotes, and for whom 21 procedures (87.5%) were considered successful. In the 14 remaining subjects—all carriers of 4G—8 procedures (57.1%) were considered successful ($P=.05$) (Table 3).

Multivariate logistic regression showed four variables to be independent risk factors for STEMI: carrying the 4G allele (genotypes 4G/4G and 4G/5G), smoking, a family background of cardiovascular disease, and high blood pressure (Table 4).

The highest concentrations of PAI-1 were detected in the subjects with the 4G/4G genotype (74.8 [11.7] ng/100 mL); those with the 4G/5G genotype had a mean value of 51.2 (10.9) ng/mL, and those with the 5G/5G genotype had the lowest mean value at 39.2 (7.74) ng/mL ($P<.001$). In association analysis, PAI-1 concentrations of the third tertile (ie, the highest concentrations) were found to be associated with high blood pressure ($P=.05$), smoking ($P=.02$), and possession of the 4G allele ($P=.01$). In logistic regression analysis, possession of the 4G allele (OR=5.52; 95% CI, 2.08-17.05; $P=.001$) and smoking (OR=5.05; 95% CI, 1.82-12.24; $P=.002$) were the only variables associated with high levels of PAI-1 and STEMI.

DISCUSSION

The etiology of cardiovascular disease is multifactorial but strongly involves genetic and environmental factors. An increase in PAI-1 in vulnerable atherosclerotic plaques associated with an increased inflammatory response might provide the necessary conditions for an atherothrombotic event—such as STEMI—in young patients in whom initially non-obstructive lesions are expected. Some authors have reported an association between 4G/5G polymorphism in the promoter region of PAI-1 and the development of AMI,^{9,17,18} but the

TABLE 3. Clinical Impact of the Different Alleles in the Present Patients With STEMI

	Allele 4G	Allele 5G	P
Estimation of size of infarction			
LEVF, %	44.7 (8.5)	44.3 (9.6)	.58 ^a
Creatine kinase, U/L	1756 (1661)	956 (767)	.01 ^a
Troponin, ng/dL	15.6 (13.08)	8.5 (8.4)	.05
Inflammatory status			
Leukocytes/ μ L	10 754 (2232)	10 436 (3113)	.64 ^a
Fibrinogen, mg/dL	585 (187)	471 (133)	.02 ^a
Type of reperfusion			
PTCA performed, n	14	24	
PTCA deemed successful, n (%)	8 (57.1)	21 (87.5)	.05 ^b

LEVF indicates left ventricular ejection fraction; PTCA, percutaneous transluminal coronary angioplasty.

^aStudent *t* test.

^b χ^2 test.

TABLE 4. Adjusted Odds Ratio for Myocardial Infarction Associated With Possession of the 4G Allele and Other Risk Factors

Variable	OR (95% CI)	P
Diabetes mellitus	1.26 (0.40-3.97)	.69
Dyslipidemia	0.87 (0.27-2.77)	.82
Allele 4G	2.29 (1.12-4.68)	.022
High blood pressure	5.42 (1.67-17.56)	.005
Smoking	23.23 (8.92-60.47)	<.001
Family background of coronary heart disease	4.66 (2.06-10.52)	<.001

results of others have failed to corroborate this.^{12,15} To our knowledge this is the first study involving the Mexican population to assess the participation of 4G/5G polymorphism in STEMI in patients ≤ 45 years of age.

In the majority of populations around the world, the 4G allele appears with greater frequency than the 5G allele. However, among the control subjects in the present study the allelic frequency of 4G was just 28%, one of the lowest reported but similar to that indicated for the African American²⁴ (25%) and Japanese (30%)³¹ populations. The low frequency of the 4G allele in the present control group agrees with the findings of Ruiz-Quezada et al.²³ The study population of the present work (Mexican mixed race) is a mixture of indigenous American, black and Spanish, which might explain the difference in allelic frequency compared to other populations.

In agreement with previously reported findings,⁸⁻¹² the present results show that the 4G allele is a risk factor for STEMI. However, they differ from those reported for other young populations,^{32,33} or which

showed this genotype to be a risk factor only among those who smoked.²²

Variability in PAI-1 plasma concentrations has been reported in different ethnic groups around the world.^{5,7,14} In some cases this appears to be governed by 4G/5G polymorphism¹⁴ while in others environmental factors such as smoking are involved³⁴ along with certain components of metabolic syndrome such as dyslipidemia, obesity and the insulin concentration,³⁵ or the interaction between smoking and this syndrome^{36,37} which increase the risk of cardiovascular disease. Festa et al²⁵ reported the ethnic differences in the distribution of 4G/5G polymorphism to be a determining factor in the plasma concentration of PAI-1. In the present work, the 4G/4G homozygous subjects showed the highest plasma concentrations of PAI-1, the lowest were seen in subjects with the 5G/5G genotype, and intermediate concentrations were recorded in heterozygotes. However, interaction with other, traditional risk factors is almost certainly involved in the development of a STEMI and it is important to identify them if primary prevention from early in life is to be improved. Smoking by patients carrying the 4G allele may have an important impact on the frequency of AMI. Anti-tobacco campaigns aimed at this group should therefore be intensified and the screening of such individuals should be contemplated.

In the present work, plasma PAI-1 concentrations measured six weeks post-STEMI were highest in those subjects with a 4G allele, as reported by Serrano Ríos et al³⁸ in patients with metabolic syndrome. Such increases in PAI-1 have been associated with AMI, and although these concentrations progressively fall Panahloo et al report that they can remain high for 6 months.³⁹ These findings agree with the proposal of Sobel et al⁴⁰ that the overexpression of PAI-1 leads to a reduced smooth muscle fiber content in atherosclerotic plaques, inducing a reduction in the amount of collagen and extracellular matrix proteins, a reduction in resistance to atheroma, the development of a vulnerable plaque, and its eventual breakage and consequent AMI. Shindo et al⁴¹ reported a significant increase in the expression of PAI-1 in the atheromas of patients who suffered an AMI compared to those with stable angina, suggesting an increased concentration of PAI-1 may be one of the factors leading to AMI. Further, an increased concentration of PAI-1 favors a state of hypofibrinolysis via the inhibition of tPA and therefore a reduction in the transformation of plasminogen into plasmin, a key enzyme in the regulation of the fibrinolytic system.⁴

It might therefore be hypothesized that the 4G allele is associated with high concentrations of PAI-1 and accordingly with two mechanisms that

favor the onset of an AMI: the formation of vulnerable plaques and a reduction in fibrinolysis. This could be of particular interest in explaining the physiopathological mechanisms behind STEMI in young patients.

CONCLUSIONS

Along with the traditional risk factors, the 4G allele is an independent risk factor for the appearance of STEMI in patients under 45 years of age. The detection of this allele along with other risk factors may therefore be useful in primary prevention. Subjects who carry the 4G allele have higher PAI-1 concentrations, which might be involved in events leading to STEMI.

ACKNOWLEDGMENTS

The authors are grateful for the economic support of the Fondo para el Fomento de la Investigación FOFOI-IMSS of Mexico (FP 2005/1/I/174 para I.I.S.), (2005/6/1/444 para I.I.S.), and the Secretaría de Investigación y Posgrado (SIP) 20060622.

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