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

Irma Isordia-Salas, Alfredo Leaños-Miranda, Irma M. Sainz, Elba Reyes-Maldonado, and Gabriela Borrayo-Sánchez

4G/5G Polymorphism With ST Elevation Acute Myocardial Infarction in Young Patients

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. Thrombosis. Plasminogen activator inhibitor-1. Coagulation.

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.
INTRODUCCIÓN

La enfermedad cardiovascular es el importante causa de morbilidad y mortalidad a nivel mundial, y la infarto miocárdico con elevación de ST (STEMI) es la causa más común de muerte en México.1 Sobre el 9% de los eventos ocurre en sujetos menores de 45 años; se estima que un genético es involucrado en algunos 20%–60% de estos casos.2

Certamente, se sabe que la actividad de la fibrinólisis está disminuida en los pacientes menores de 45 años que sufren un infarto miocárdico agudo (AMI).3 Plasminogen activator inhibitor type 1 (PAI-1) es el inhibidor físico de la actividad de la fibrinolítica del sistema. Se logra esto a través de la inhibición de la activación plaquemática (tPA) y a través de la inhibición de la actividad del activador urinoclástico (uPA). Un incremento en la concentración plasmática es asociado con eventos trombóticos.4 El polimorfismo consiste en una inserción/delección de una base de guanina en la posición 675 en el promotor del PAI-1 que da lugar a 2 alelos—4G y 5G—que difieren en su regulación de la concentración del PAI-1.5,6 Individuos que son homocigotos para el alelo 4G (4G/4G) tienen concentraciones de PAI-1 más altas que aquellos que son homocigotos para 5G (5G/5G). Los individuos que son heterocigotos (4G/5G) tienen niveles intermedios.7 El alelo 5G presenta un sitio de unión adicional de un inhibidor que está ausente en el alelo 4G; como consecuencia, este 4G alelo produce hasta 6 veces más mARN y está asociado con un mayor PAI-1 actividad.

La información disponible sobre el riesgo de 4G/5G polimorfismo en el desarrollo de AMI es contradictorio. Algunos estudios han mostrado una asociación entre este polimorfismo y un incremento del riesgo de STEMI,8,10,11 mientras que otros sugieren que el riesgo se reduce13 o no existe asociación en absoluto.14-16 Un incremento en la concentación plasmática de PAI-1 es, sin embargo, asociado con mayor mortalidad y el sufrimiento de un segundo infarto en pacientes menores de 45 años.8

Diferentes poblaciones alrededor del mundo muestran variación en términos de las frecuencias alélicas del 4G y 5G, con el 4G alelo más común en Asia (59%),10,11,14,15 en los blancos (51%),10,11,14,15 en los hispanos (47%),14,16 y en los indios (54%).22 Las poblaciones más como las populaciones de indios, los Españoles (47%) y los afroamericanos (34%)21 presentan un riesgo inferior (20%-25%) respecto a los africanos (1%-5%).23

El objetivo del presente trabajo fue determinar la asociación entre el transporte de 4G o 5G alelos, el PAI-1 producido, y el desarrollo de STEMI en pacientes mexicanos mayores de 45 años.

MÉTODOS

Los pacientes STEMI incluidos en este estudio fueron consecutivos que fueron admitidos a la unidad de cuidado cardíaco del Hospital de Cardiología del Centro Médico Nacional Salud XXI durante el periodo de enero de 2006-March 2007. Los pacientes incluidos fueron pareados con controles según edad y sexo.

El criterio para el diagnóstico de STEMI (ESC/AHA/ACC)8,28 fue dolor torácico que duró más de 20 min o más, la elevación de MB creatina kinase (CK-MB) o troponinas hasta el percentil 99 o mayor, y la elevación del ST segmento en 2 leads en 2 segmentos contiguos en V1 y V3 y por 1 mm en los leads restantes.

Los pacientes dieron su consentimiento informado. El protocolo de estudio fue aprobado por el comité de Ética y Investigación del Instituto Mexicano del Seguro Social en conformidad con las reglas del Comité de Ética y Investigación del Instituto Mexicano del Seguro Social en conformidad con el Código de Ética de Helsinki.

Los controles probados eran donantes de sangre sanos que asistieron al mismo hospital.

Diabetes mellitus: sujetos con un nivel de azúcar en ayunas superior a 126 mg/dL o en tratamiento para la diabetes.

Dyslipidemia: sujetos con un nivel de colesterol superior a 200 mg/dL o en tratamiento para la dyslipidemia.


ABREVIATURAS

PAI-1: Plasminogen activator inhibitor type 1
STEMI: ST elevation myocardial infarction
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). 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 94ºC for 3 min followed by 30 cycles of denaturation at 94ºC for 30 s, alignment at 60ºC for 30 s, and an extension step at 72ºC for 30 s, followed by a final linear extension step at 72ºC 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 55ºC. 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.

**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 4ºC to avoid the contamination of the plasma with platelets. They were then stored in aliquots of 0.5 mL at −70ºC until use. The plasma concentration of PAI-1 was determined immunoenzymatically by ELISA (Colilza 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). 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 94ºC for 3 min followed by 30 cycles of denaturation at 94ºC for 30 s, alignment at 60ºC for 30 s, and an extension step at 72ºC for 30 s, followed by a final linear extension step at 72ºC 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 55ºC. 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.

**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).
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 \((OR=1.77; 95\% \text{ CI}, 1.04-3)\) (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 (5.78 [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)\(/\mu L\) and 10 436 (3113) \(/\mu L\) \((P=.64)\) for the 5G homozygotes. Percutaneous transluminal coronary angioplasty (PTCA) was performed in 38 patients, 24 of whom were 5G.

### 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 \(\chi^2\) 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 \(\leq 45\) 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 \((OR=12.64; 95\% \text{ CI}, 6.46-25)\), high blood pressure \((OR=5.63; 95\% \text{ CI}, 2.68-12.01)\), diabetes mellitus \((OR=5.58; 95\% \text{ CI}, 2.52-12.63)\), dyslipidemia \((OR=5.79; 95\% \text{ CI}, 2.69-12.68)\), and a family background of cardiovascular disease \((OR=5.52; 95\% \text{ CI}, 2.78-11.09)\). Table 1 shows the distribution of the localization of AMI.

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

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

AMI indicates acute myocardial infarction; BMI, body mass index.
*Student t test.
*\(\chi^2\) test.
Values are means (SD) unless otherwise indicated.

Patients (n=127) Controls (n=127)

<table>
<thead>
<tr>
<th>Allelic frequency, n (%)</th>
<th>Genotype, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4G</td>
<td>5G/5G 54 (42.5) 5G/5G 54 (42.5)</td>
</tr>
<tr>
<td>5G</td>
<td>4G/5G 64 (50.4) 4G/5G 64 (50.4)</td>
</tr>
<tr>
<td></td>
<td>4G/4G 9 (7.1) 4G/4G 9 (7.1)</td>
</tr>
<tr>
<td>Allelic frequency, n (%)</td>
<td>Genotype, n (%)</td>
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<td></td>
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PAI-1 indicates plasminogen activator inhibitor-1.

**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, but the results of others have failed to corroborate this.

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 American (25%) and Japanese (30%) populations. The low frequency of the 4G allele in the present control group agrees with the findings of Ruiz-Quezada et al. 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, the present results show that the 4G allele is a risk factor for STEMI. However, they differ from those reported for other young populations, or which...
showed this genotype to be a risk factor only among those who smoked.\textsuperscript{22}

Variability in PAI-1 plasma concentrations has been reported in different ethnic groups around the world.\textsuperscript{5,7,14} In some cases this appears to be governed by 4G/5G polymorphism\textsuperscript{44} while in others environmental factors such as smoking are involved\textsuperscript{34} along with certain components of metabolic syndrome such as dyslipidemia, obesity and the insulin concentration,\textsuperscript{35} or the interaction between smoking and this syndrome\textsuperscript{36,37} which increase the risk of cardiovascular disease. Festa et al\textsuperscript{38} 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 and others environmental factors such as smoking are involved\textsuperscript{34} along with certain components of metabolic syndrome such as dyslipidemia, obesity and the insulin concentration,\textsuperscript{35} or the interaction between smoking and this syndrome\textsuperscript{36,37} which increase the risk of cardiovascular disease. Festa et al\textsuperscript{38} 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 et al\textsuperscript{39} 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.\textsuperscript{40} These findings agree with the proposal of Sobel et al\textsuperscript{41} 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 al\textsuperscript{42} 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.\textsuperscript{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.

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