The PAI-1 Gene Locus 4G/5G Polymorphism Is Associated With a Family History of Coronary Artery Disease

Maurizio Margaglione, Giuseppe Cappucci, Donatella Colaizzo, Nicola Giuliani, Gennaro Vecchione, Elvira Grandone, Orazio Pennelli, Giovanni Di Minno

Abstract—A family history of ischemic events is a major determinant of coronary artery disease (CAD). Plasma levels of plasminogen activator inhibitor 1 (PAI-1) modulate this risk. A deletion/insertion polymorphism within the PAI-1 locus (4G/5G) affects the expression of this gene. We investigated the relationship between the PAI-1 4G/5G polymorphism in 1179 healthy employees of our institution and the occurrence of CAD in their first-degree relatives. A family history of documented ischemic coronary disease was assessed by a modified WHO questionnaire. The PAI-1 4G/5G polymorphism was evaluated by polymerase chain reaction and endonuclease digestion. The group with a first-degree relative who had suffered from a coronary ischemic episode had a higher number of homozygotes for the deleted allele (4G/4G) of the PAI-1 gene compared with subjects without such a family history (odds ratio [OR]=1.62, 95% confidence interval [CI]=1.17 to 2.25; P=.005). The frequency of the 4G allele was abnormally high as well (OR=1.29, 95% CI=1.04 to 1.60; P=.025). The individuals with a positive family history were older (P<.001) and exhibited a higher body mass index (P=.033) and total cholesterol levels (P<.001) than those without. In a multiple logistic regression analysis, age (P=.006) and PAI-1 4G/4G (P=.024) independently contributed to a family history of coronary heart disease, with 4G/4G carriers exhibiting a more frequent family history of CAD (OR=1.60). The PAI-1 4G/5G polymorphism to some extent thus accounts for the risk of CAD related to a family history for such an event. These findings support the hypothesis that the 4G variant is a transmissible coronary risk factor. **(*Arterioscler Thromb Vasc Biol*. 1998;18:152-156.)**

Key Words: coronary disease ■ genes ■ risk factors

In Western countries, the ischemic complications of atherosclerosis, ie, acute MI and ischemic stroke, are the most common causes of morbidity and mortality.[1] It is known that individual variation or environmental factors, such as overweight, diabetes mellitus, arterial hypertension, cigarette smoking, etc, are associated with a high risk for MI.[2] The development of myocardial ischemia can only partially be ascribed to the classic risk factors. In the 26 countries of the WHO/MONICA Core Study, up to 20% of the difference in mortality for CAD among the various populations was correlated with the coexistence of hypertension, dyslipidemia, and smoking.[3] Differences in lifestyle, such as diet composition, smoking habits, stress, and obesity may only partially reflect changes in the incidence rate.

In recent years, epidemiological studies have shown that abnormalities in some hemostatic parameters may help predict the risk for ischemic events. An increased risk for arterial thrombosis has been associated with high plasma levels of coagulation and fibrinolytic factors.[4–6] Low fibrinolytic activity is related to raised plasma levels of PAI-1 and has been documented in subjects who will develop MI.[7,8]

In population studies, a family history of ischemic coronary events is a major predictor of CAD. The familial aggregation of CHD can be accounted for in large part by the clustering of cardiovascular disease risk factors. It is well recognized that a history of parental CHD is associated with increased risk of myocardial ischemia.[9] The presence of CAD in a first-degree relative before the age of 55 increases by 10-fold the risk to other family members.[10] In several twin studies, most showed a strong genetic component in the pathogenesis of cardiovascular ischemia.[11,12] These findings support the hypothesis that genetic factors play a significant role in MI and vascular risk factors.[13]

Recently, raised PAI-1 plasma levels have been shown to be related to a single-base-pair guanine deletion/insertion (4G/5G) polymorphism.[14,15] Evidence is accumulating that though not sufficient, this variation may enhance the coronary risk (susceptibility locus).[16] Previous work has documented that Lp(a) plasma levels and a polymorphism of the angiotensin-converting enzyme gene are independently associated with a parental history of MI and CHD in second-degree relatives.[17–19] Little is known of PAI-1 gene variants and how they

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Selected Abbreviations and Acronyms

CAD = coronary artery disease
CHD = coronary heart disease
CI = confidence interval
MI = myocardial infarction
OR = odds ratio
PAI-1 = plasminogen activator inhibitor 1

might be related to such a familial history. To address this issue, we have carried out an investigation in a cohort of healthy workers from southern Italy.

Methods

Subjects

From January 1995 to December 1996, we interviewed 1272 apparently healthy employees of the Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, southern Italy. Of these, 66 refused to participate and blood specimens for 24 could not be typed. Three subjects, all men, had documented evidence of CHD after their enrollment and were excluded from the analysis. Thus, 1179 subjects (22 to 66 years old) were enrolled. All subjects were white, and all of their parents and grandparents had been born in the same region. The male-to-female ratio was 0.77 (males=552, 43.4%; females=720, 56.6%). A complete clinical summary with emphasis on personal and family histories for angina pectoris, MI, ischemic stroke, peripheral arterial disease, and vascular risk factors was obtained from all subjects by a specially trained staff, who utilized a previously described questionnaire.[20] This questionnaire, in addition to the detailed and specific questions about symptoms of ischemic heart disease, peripheral vascular disease, and previous vascular surgery from the WHO questionnaire for cardiovascular disease, contains specific questions concerning stroke. Additional questions were asked concerning arterial hypertension, dyslipidemia, drugs taken, alcohol use, and smoking habits. After approval of the study protocol by the local ethics committee, the study was carried out according to the Principles of the Declaration of Helsinki; informed written consent was obtained from all subjects.

Detection of Biochemical and Genetic Variables

Plasma levels of PAI-1 antigen, total cholesterol, and triglycerides were determined as described elsewhere.[20] Blood samples were collected and DNA extracted according to standard protocols.[21] The PAI-1 4G/5G polymorphism was evaluated as described.[22] In brief, a mutated oligonucleotide was synthesized, which inserts a site for the *Bsl* I enzyme within the product of amplification. PCR was carried out on 50-μL-volume samples in a Perkin Elmer-Cetus thermal cycler. Each sample contained 0.5 μg of genomic DNA, 15 pmol of each primer, 100 mmol/L of dNTP, 10 mmol/L Tris HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, and 1 U thermostable *Taq* polymerase. The 30 cycles consisted of steps at 95°C for 1 minute, 60°C for 1 minute, and 72°C for 2 minutes. Then 20-μL volumes of the amplification products were digested for 2.5 hours at 55°C with 5 U of the *Bsl* I restriction enzyme. The fragments were fractionated by 4% agarose-gel electrophoresis and visualized under UV light.

Statistical Analysis

All analyses were performed according to the Systat 5.2.1 statistical package.[23] Differences in baseline characteristics between groups with different ischemic family history were evaluated with Mann-Whitney and χ^2 tests for continuous and discrete variables, respectively. The allelic frequencies were estimated by gene counting and genotypes were scored. The observed numbers of each PAI-1 genotype were compared with those expected for a population in Hardy-Weinberg equilibrium by using a χ^2 test. The significance of the differences of observed alleles and genotypes between groups was tested using the χ^2 analysis. ORs and 95% CIs were calculated. Statistical significance for differences in continuous variables among PAI-1 genotypes was tested by univariate ANOVA. Pairwise multiple comparisons were performed using Scheffé's method after logarithmic transformation of variables with high skewness. Appropriate models were established to evaluate in a logistic regression analysis the independent relationship of the PAI-1 4G/5G polymorphism with the family history of coronary ischemic disease. Adjusted ORs and 95% CIs were calculated with logistic regression models. Statistical significance was taken at $<.05$.

Results

Table 1 shows demographic characteristics of the study group analyzed as a whole and stratified according to family history of CHD. The two groups differed with respect to age ($P<.001$), cholesterol ($P<.001$), and body mass index ($P=.033$) but not with respect to PAI-1 plasma levels ($P=.214$). Genotype frequencies in the entire sample were .26 (4G/4G; n=307), .49 (4G/5G; n=578), and .25 (5G/5G; n=294) and were similar to those observed in other white populations.[14,15] The

TABLE 1. Characteristics of the Whole Group, According to Familial History of Myocardial Ischemia

Parameters	Total (n=1179)	Subjects With a First-Degree Relative Affected (n=198)	Subjects Without a First-Degree Relative Affected (n=981)	Significance (P)*
M/F%, (n)	0.77 (512/667)	0.77 (86/112)	0.77 (426/555)	NS†
Age median, (range in years)	36 (22-66)	40 (22-65)	35 (22-66)	<.001
Hypertension, % (n)	2.46 (29/1150)	3.1 (6/192)	2.3 (23/958)	NS
Diabetes mellitus, % (n)	1.61 (19/1160)	2.5 (5/193)	1.4 (14/967)	NS
Current smokers, % (n)	23.7 (279/900)	26.3 (52/146)	23.1 (227/755)	NS
Alcohol drinkers, % (n)	55.0 (649/530)	57.6 (114/84)	54.5 (535/446)	NS
BMI‡, kg/m^2	24.29 (3.59)	24.87 (3.79)	24.17 (3.54)	.033
Cholesterol, mmol/L	4.87 (0.99)	5.10 (0.99)	4.81 (0.98)	<.001
Triglycerides, mmol/L	1.28 (0.88)	1.28 (0.85)	1.28 (0.89)	NS
PAI-1, ng/mL	15.90 (11.55)	16.93 (11.70)	15.69 (11.52)	NS

*χ^2 test for categorical and Mann-Whitney *U* test for continuous variables were used. For continuous variables means (standard deviation) are indicated.
†NS, indicates not significant.
‡BMI, indicates body mass index.

TABLE 2. PAI-1 4G/5G Genotype in Subjects, According to Familial History of Myocardial Ischemia*

	Subjects With a First-Degree Relative Affected		Subjects Without a First-Degree Relative Affected	
Genotype	**n**	**%**	**n**	**%**
4G/4G	68	34.34	239	24.36
4G/5G	85	42.93	493	50.26
5G/5G	45	22.73	249	25.38
Total	198	100.00	981	100.00

*$\chi^2_{2\ def.}$: 8.571; P=.014

frequencies observed were not significantly different from those predicted by Hardy-Weinberg equilibrium. When stratified according to family history (Table 2), individuals whose first-degree relatives suffered from coronary ischemic disease showed a significantly higher frequency of the 4G allele (n=221, 55.81%) of the PAI-1 gene polymorphism compared with that (n=971, 49.49%) observed in subjects without such family history (χ^2=5.012, OR=1.29, 95% CI=1.04 to 1.60; P=.025). Different frequencies between the two subsets were also observed (Table 2) when the genotypes were analyzed. Furthermore, if one assumes a recessive (4G/4G versus [4G/5G and 5G/5G]) effect of the 4G allele, then the frequency of the 4G/4G genotype was significantly higher in individuals with at least one affected first-degree relative than in those without (χ^2=8.521, OR=1.62, 95% CI=1.17 to 2.25; P=.004). Additional analysis assuming a dominant ([4G/4G and 4G/5G] versus 5G/5G) effect of the 4G allele failed to show any significant difference (χ^2=0.620; P=.431).

When the entire sample was analyzed according to the PAI-1 gene polymorphism (Table 3), there was no difference with respect to a series of determinants of PAI-1 plasma levels (eg, age, hypertension, diabetes mellitus, etc). In contrast, mean plasma PAI-1 concentration varied significantly among the different genotypes (ANOVA test: F=7.897; P<.001), being higher in 4G/4G subjects (17.97±13.32 ng/mL) than in 4G/5G and 5G/5G subjects (15.66±11.49 ng/mL, P=.021; 14.22±9.17 ng/mL, P<.001, respectively).

To assess the relationship of PAI-1 with a family history of CAD, multiple regression analysis models were employed. In a model in which a recessive (4G/4G versus [4G/5G and 5G/5G]) effect of the 4G allele was assumed, PAI-1 4G/5G polymorphism (P=.024) and age (P=.006), but not blood cholesterol (P=.089) or body mass index (P=.550), were significantly and independently related to a history of a first-degree relative with CAD. The adjusted OR for carrying the 4G/4G alleles was 1.60 (95% CI=1.06–2.37). The inclusion of PAI-1 antigen levels in the model little affected the strength of the association with 4G/4G (P=.017). The value for the −2 log likelihood with PAI-1 antigen levels was 29.11 (*df* 5), only slightly less than that for the model without (29.85, *df* 4). In parallel, the −2 log likelihood statistics of the model without (971.6) was higher than that of the model with PAI-1 antigen levels (954.9). These values differ significantly (difference in −log likelihood=16.6; P<.001). Models that included a dominant ([4G/4G and 4G/5G] versus 5G/5G) or an additive (4G/4G versus 4G/5G versus 5G/5G) effect of the 4G allele did not show a significant association of the PAI-1 4G/5G polymorphism with a history of a first-degree relative with CAD (P=.593 and .091, respectively).

Discussion

The PAI-1 4G/5G polymorphism has been suggested to exert a role in myocardial ischemia, with 4G/4G genotypes carrying the highest circulating levels of PAI-1.[14,15] The possibility of a correlation between PAI-1 genotype and a positive familial history has not been addressed so far. We reasoned that if the distribution of the 4G/4G genotype was increased in subjects with coronary ischemic disease, then their relatives should have this genotype more often than the relatives of unaffected individuals. Our data support such a possibility in first-degree relatives (Table 2). The two subsets also differed with respect to age, mean cholesterol levels, and body mass index, all of these variables being higher in subjects with a family history of CAD (Table 1). In the attempt to assess whether the relationship

TABLE 3. Clinical (and Biochemical) Characteristics According to PAI-1 4G/5G Polymorphism*

Parameters	**4G/4G (307)**	**4G/5G (578)**	**5G/5G (294)**	**Significance (*P*)**
M/F, % (n)	0.75 (132/175)	0.79 (256/322)	0.73 (124/170)	NS†
Age median, (range in years)	37 (23-65)	36 (22-66)	36 (22-62)	NS
Hypertension, % (n)	2.9 (9/298)	2.1 (12/566)	2.7 (8/286)	NS
Diabetes mellitus, % (n)	1.3 (4/303)	1.4 (8/570)	2.4 (7/287)	NS
Smokers, % (n)	24.4 (75/232)	24.0 (139/439)	22.1 (65/229)	NS
Alcohol drinkers, % (n)	58.3 (179/128)	54.0 (312/266)	53.7 (158/136)	NS
BMI, kg/m^2	24.21 (3.67)	24.27 (3.59)	24.41 (3.53)	NS
Cholesterol, mmol/L	4.95 (0.94)	4.85 (1.03)	4.86 (0.96)	NS
Triglycerides, mmol/L	1.33 (0.91)	1.26 (0.84)	1.32 (0.96)	NS
PAI-1, ng/mL	17.97 (13.32)‡	15.66 (11.49)	14.22 (9.17)	<.001

*χ^2 test for categorical and ANOVA test for continous variables were used. Skewed variables were log transformed and arithmetical means standard deviation are indicated.

†NS, indicates not significant.

‡P<.001 vs. 5G/5G; P=.021 vs. 4G/5G (Scheffé's test).

between 4G/4G genotype and family history was independent of other variables, a multiple logistic regression model was performed, which included other significant covariates in addition to the PAI-1 4G/5G polymorphism.. Findings from this statistical analysis showed a significant excess (adjusted OR=1.60) of a family history of CAD in 4G/4G carriers, confirming the strength of the association observed in univariate analysis (crude OR=1.62). Alternative multiple logistic models that assumed a different gene effect (ie, additional or dominant) of the 4G allele did not show any significant relationship between the PAI-1 4G/5G polymorphism and family history of CAD. Entering plasma PAI-1 antigen levels into the model did not significantly affect the association between the 4G/4G genotype and family history of CAD. In addition, the change in −2 log likelihood statistics between the two recessive models showed that the model without PAI-1 antigen levels improved the ability to explain the coronary ischemic risk related to a positive family history.

Our findings differ from those of the US Physicians' Health Study,[24] in which no association between PAI-1 genotype and MI was found. Although the inconsistency may reflect the play of chance, alternative explanations have to be considered. First is the possibility of differences in genetic background. We enrolled subjects from southern Italy whose families have resided in the region for at least two generations and whose grandparents were born in Italy. In the present report the two groups with a different family history of CAD share similar rates of hypertension and diabetes mellitus, and the association with the PAI-1 4G/5G polymorphism was checked for other risk factors in which the groups differed. Furthermore, the hypothesis that our subjects belong to a genetic subgroup that, though white, might have a segregated PAI-1 genotype with a CAD phenotype must be taken into account. However, the frequencies of several alleles of the major histocompatibility complex in our study population (A2, 26.3%; DR7, 12.0%; and DRw8, 2.3%) were not different from those reported in whites (A2, 28.9%; DR7, 12.0%; and DRw8, 3.0%).[25]

As reported,[14,15] mean plasma levels of PAI-1 antigen were different in the three genotypes ($P<.001$). However, these levels were not significantly higher in subjects with a family history of CAD (Table 1). This finding may be explained by taking into consideration that in addition to genotype, a series of environmental factors affects plasma PAI-1 levels. PAI-1 production has been related to high blood levels of glucose, insulin, and triglycerides, the common link possibly being insulin resistance.[26,27] PAI-1 antigen has been shown to correlate significantly with insulin resistance as measured by a hyperinsulinemic euglycemic clamp.[28] In addition, environmentally influenced factors such as triglycerides have been reported to interact with the PAI-1 4G/5G polymorphism to regulate plasma PAI-1 levels.[29,30] Furthermore, a relationship between smoking habits and degree of insulin resistance as well as higher PAI-1 activity has been found.[31] As illustrated (Table 1), the incidence of diabetes mellitus, arterial hypertension, mean levels of triglycerides, and the percentage of current smokers were not different between the two groups.

In the Tromsø Heart Study, there was 78% agreement between a self-reported history of MI in first-degree relatives and physicians' records, hospital records, and death certificates.[32] Such agreement was >86% in an Australian Study.[33] In the Tecumseh Community Health Study[34] as well as in the study by Badenhop et al,[19] underreporting of coronary events was more likely than overreporting. In our study, information was collected by a well-trained staff and was limited to definite coronary ischemic events according to the WHO questionnaire. This questionnaire has a specificity and sensitivity of 91% and 81%, respectively, for angina pectoris, 91% and 87% for MI, and 100% and 92% for intermittent claudication.[35] Our study population is living in a very restricted area. For the past 40 years, the hospital where the offspring are employed has been the reference health institution for the large majority of subjects living in the area. Thus, any inaccuracy would tend to lower rather than enhance the risk estimates of a positive family history.

One should also consider that our calculated ORs reflect only the association between the PAI-1 4G/4G genotype and a family history of CAD. Despite "dilution" of the genes, the association between poor fibrinolysis and ischemic risk confers a biological plausibility to our findings. However, the possibility cannot be definitively ruled out that the PAI-1 4G/5G polymorphism might be a neutral marker in tight linkage disequilibrium with a functional variant of a sequence yet to be identified. Our data support the concept that the PAI-1 gene is a susceptibility locus, ie, it is neither necessary nor sufficient for the disease to occur, but makes it more likely that one will become ill. The extent to which this polymorphism confers an additional coronary risk has to be addressed in prospective studies.

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