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REVIEW ARTICLE

The effect of the plasminogen activator inhibitor-1 4G/5G polymorphism on the thrombotic risk

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Abstract

Plasminogen activator inhibitor (PAI-1), is the central component of the fibrinolytic system. A deletion/insertion (4G/5G) polymorphism in the promoter region of the PAI-1 gene has been correlated with levels of plasma PAI-1. The 4G allele is associated with higher levels of PAI-1, and might increase the risk for intravascular thrombosis. However, the contribution of this genetic variant to the risk for thrombosis, both arterial and venous, has not been clearly established. A broad spectrum of findings regarding the effect of the 4G allele on thrombotic risk in different target organs has been reported. Our aim is to summarize the variable influence of this polymorphism on thrombotic events in different tissues or organs and explain the underlying mechanisms accounting for these differences.

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Introduction

An altered balance between the fibrinolytic and procoagulant systems might significantly contribute to the pathophysiology of thrombus formation. This hemostatic imbalance may be the result of an impaired fibrinolytic action that in combination with either enhanced procoagulant or defective anticoagulant mechanisms, increases the chances for thrombotic events to occur. During fibrinolysis, plasminogen is converted to plasmin, which in turn dissolves fibrin clots. This conversion occurs in the presence of urokinase plasminogen activator (u-PA), or tissue plasminogen activator (t-PA) and is regulated to a significant extent by the serine protease inhibitor, plasminogen activator inhibitor-1 (PAI-1) [1] (Fig. 1). Because of its major role in the regulation of the fibrinolytic process, PAI-1 over-expression may compromise normal fibrin clearance mechanisms and promote pathological fibrin deposition and thrombotic events [2].

PAI-1 is synthesized in various tissues and cell types including liver, spleen [3], adipocytes [4], and endothelial cells. Its synthesis is regulated by various agents including insulin [5], lipids [6], glucose [7], endotoxin and inflammatory cytokines [8]. The human PAI-1 gene is located at chromosome 7q22 [9]. A genetic polymorphism of this gene has been identified in the promoter region, where one allele has a sequence of four guanosines (4G) and

the other has five guanosines (5G) at -675 bp upstream from the mRNA initiation point [10]. Both the 4G and 5G alleles have a binding site for an activator of transcription. However, the 5G allele has an additional binding site for a repressor, leading therefore to lower transcription rates and less PAI-1 activity [11]. Thus, the 4G allele has been linked with moderately higher plasma PAI-1 levels [12,13]. However, the nature of this polymorphism can be more accurately described as response polymorphism, since PAI-1 is considered a strong acute-phase reactant. This means that different PAI-1 levels between 4G and 5G are more apparent in the presence of environmental and/or disease factors, which stimulate PAI-1 expression [14].

Data regarding the effect of this genetic variant to the risk for thrombosis, both arterial and venous, are numerous and contradictory. Three meta-analyses have recently been published about the contribution of the 4G/5G polymorphism to cardiovascular, ischemic stroke and venous thromboembolism risk [15-17]. The results of these studies are presented in Table 1. Based on these latest findings, our aim is to summarize the variable influence of this polymorphism on thrombotic risk in different target organs and attempt to explain the underlying mechanisms accounting for these differences.

The relation of the PAI-1 4G/5G polymorphism with cardiovascular disease

A meta-analysis of 9 studies showed a 20% increased risk of myocardial infarction attributed to the 4G/4G genotype [18]. A weaker positive association has also been reported by a recent meta-analysis including 37 studies which yielded a per-allele relative risk of about 1.06 for coronary disease in subjects with the -675 4G variant [15]. However, this finding might merely be an artifact of selective publication. Thus, the question that still remains is how strong is the predictive value of this polymorphism for coronary artery disease or MI, especially after adjustment for other cardiovascular risk factors like blood lipids, diabetes, hypertension, obesity and smoking.

The 4G allele is known to be associated with higher PAI-1 antigen levels, which, in turn, are correlated

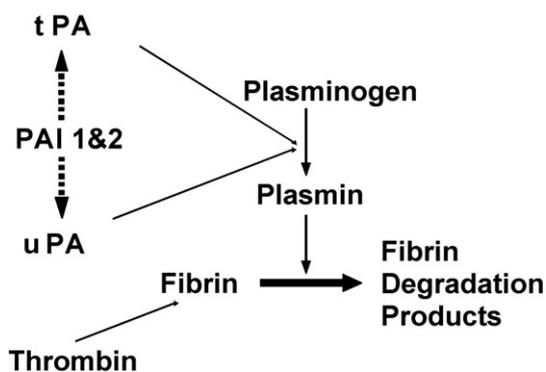


Figure 1 The role of PAI-1 in the fibrinolytic pathway. u-PA; urokinase plasminogen activator, t-PA; tissue plasminogen activator.

Table 1 Reported summary odds ratios (OR) along with the 95% confidence intervals (CI) from meta-analyses regarding the relation of the PAI-1 4G/4G polymorphism with cardiovascular disease (myocardial infarction, atherosclerosis, coronary artery disease), ischemic stroke and venous thromboembolism [15,16,17]

	Number of studies	Summary OR (95% CI)
<i>4G allele vs. 5G allele</i>		
Cardiovascular disease [15]	37	1.06 (1.02–1.10)
Ischemic stroke [16]	14	0.98 (0.858–1.121)
Venous thromboembolism [17]	17	1.15 (1.068–1.246)
<i>4G4G vs. Rest</i>		
Cardiovascular disease [15]	37	1.07 (1.01–1.13)
Ischemic stroke [16]	15	0.96 (0.783–1.184)
Venous thromboembolism [17]	16	1.14 (1.004–1.297)
<i>Rest vs. 5G5G</i>		
Cardiovascular disease [15]	37	0.95 (0.787–1.134)
Ischemic stroke [16]	14	0.95 (0.787–1.134)
Venous thromboembolism [17]	17	1.28 (1.117–1.478)

with the lipid profile. Particularly, PAI-1 is positively associated with cholesterol, LDL-cholesterol, VLDL and triglyceride levels and negatively with HDL-cholesterol [6]. Moreover, the 4G/4G genotype itself, has been reported to be associated with high cholesterol levels [19]. High plasma PAI-1 levels have also been shown to be related to insulin resistance [20], while several studies have uncovered an increase in PAI-1 levels resulting from the activation of renin–angiotensin system in subjects with the 4G/4G genotype [21]. In addition, obesity is associated with increased PAI-1 levels [22].

The findings mentioned above might explain the involvement of this polymorphism in the clustering of atherothrombotic risk factors and why people with the 4G/4G genotype are at increased risk for MI [23]. Thus, the 4G allele may confer a significant risk of myocardial infarction in populations at higher risk of myocardial infarction than in low-risk populations because of gene–environment interactions. Other authors suggest that the length of time between the first signs of CAD and the occurrence of MI should be considered when the effect of the 4G/5G polymorphism to the risk for coronary disease is examined [24]. Some MI patients have a long history of stable CAD, while others suffer MI suddenly. The former patients probably have highly stenotic

lesions without precipitating thrombosis, while the latter might exhibit high thrombogenicity or impaired fibrinolytic activity. The pathogenesis of CAD is complex and polygenetic in the vast majority of patients. So, study design and patient selection might partly explain the inconsistency of results among published studies concerning 4G/5G polymorphism of the PAI-1 gene and CAD. For example, different sample sizes, individual environmental factors or the extent of additional non-hemostatic risk factors might account for the contradictory findings reported. Moreover, the criteria used to define CAD were not uniform even when the same diagnostic method was applied (i.e. the severity of stenosis in angiography), the cut-off points for many risk factors were varying considerably resulting in misclassification of subjects, control of potential cofounders was inadequate and there were several sources for identifying controls.

Results of meta-analyses should also be interpreted with caution, since the limitations of the individual studies are propagated in a meta-analysis. Thus, very large and meticulously conducted case–control studies with globally adopted case definitions, adjustment for the most important associated risk factors and provision for taking into consideration the combination of different genes' polymorphisms may allow the performance of valid comparisons and the detection of 4G allele carriers at increased risk for MI.

The relation of the PAI-1 4G/5G polymorphism with ischemic stroke

The spectrum of findings regarding the relationship between 4G/5G polymorphism of the PAI-1 gene and stroke is quite confusing. Some authors consider that the 4G/4G genotype confers an increased risk for stroke [25,26], while others have supported a neutral or even protective effect of the same genotype against cerebrovascular events [27–31]. A recent meta-analysis failed to demonstrate a significant association, neither positive nor negative, between the 4G/5G polymorphism and cerebrovascular disease [16] (Table 1). It seems that PAI-1 is implicated in both harmful and protective biological processes leading to brain infarction. The neutral role of the 4G allele in stroke, as opposed to an increase in risk of MI, may be a manifestation of the different pathogenesis of these diseases.

Rupture of an atherosclerotic plaque is considered a central event in the pathogenesis of myocardial infarction. On the contrary, in the case of stroke small vessel disease and thromboembolism (without significant underlying large vessel disease) are more

common causes of cerebral ischemia than carotid and aortic arch atherosclerosis [32]. Another interesting hypothesis focuses to the fact that all studies included in this meta-analysis examined the effect of this genotype on stroke in patients and controls under basal conditions. On the contrary, the 2 studies concerning acute disorders showed that patients with the 4G allele in the PAI-1 gene had an increased risk for ischemic stroke [33,34]. In acute disorders PAI-1 seems to act as an acute-phase reactant. Thus, it is assumed that elevation of PAI-1 levels after stressful conditions, for example severe inflammatory diseases or life-threatening disorders, in patients with the 4G allele is much more pronounced, resulting in impaired fibrinolysis. Under these circumstances, the formation of microthrombi is no longer counteracted by the fibrinolytic system.

It is known that PAI-1 is associated with many components of the metabolic syndrome. However, stroke is not so clearly related to hypercholesterolemia as coronary artery disease does and other effects of this genotype (increased PAI-1 expression) may protect against stroke. It is noteworthy that a tendency for a protective effect of the PAI-1 4G/4G genotype has been observed in many studies. Given that the 4G/4G genotype confers a high PAI-1 expression in astrocytes, it has been suggested that this might rescue neurons from the deleterious effects of t-PA, and explain the protective effect of this genotype in stroke [35]. Another potential mechanism accounting for such an effect might be the inhibition of the activation of matrix-degrading enzymes in the atherosclerotic plaque, thereby preventing rupture followed by an ischemic infarction [36]. It is postulated that despite its antifibrinolytic properties, PAI-1 may stabilize plaque and neutralize tissue plasminogen activator at inflammatory sites. A prospective study in women supports this notion [27].

It remains uncertain why the same mechanism does not confer a protective effect in MI where rupture of atherosclerotic plaques is considered a causal event. It seems that the involvement of PAI-1 in stroke is more complicated. In order to predict the variable effects of this polymorphism in several groups of patients, the underlying mechanisms and their interactions should be explored and understood. This will contribute to identification of patients in whom evaluation of this polymorphism might carry a predictive value.

The relation of the PAI-1 4G/5G polymorphism with venous thromboembolism

Data regarding the association between the presence of allele 4G and the risk for venous thrombotic

episodes (VTE) have also been controversial. Most studies have found no relationship between the -675 4G/5G polymorphism and the development of VTE in unselected patients [37–39]. However, this polymorphism has been shown to increase the risk for VTE in patients with other congenital or acquired prothrombotic disorders [40–43], although this was not a consistent finding [44–46].

A recent meta-analysis revealed an unexpected mild association of the 4G allele with risk of venous thromboembolism, when analysis focused on subjects with venous thrombosis who were not included in certain risk groups with other genetic or acquired thrombophilic defects [17] (Table 1). This finding was unexpected since most individual studies failed to demonstrate any significant relationship between this polymorphism and the development of venous thrombosis in unselected patients. Furthermore, a significant role for PAI-1 4G/5G polymorphism in the risk of thrombotic events in patients with a congenital predisposition to thrombosis was also found.

Based on these data, we can support the idea that the presence of the 4G allele might significantly increase the thrombotic risk in patients with inherited thrombophilia and to a lesser degree in cases without known risk factors. It is known that thrombophilia is a multigenic disorder, where the genetic background affects the severity and phenotypic manifestation of a certain thrombophilic state. Thus, it seems that the 4G/5G polymorphism is probably a mild risk factor which individually conveys little risk while its clinical manifestations become more evident when it is combined with other predisposing genetic risk factors. Clinical data support in a more convincing way the involvement of this polymorphism in VTE than in stroke or MI, particularly in subjects with other genetic thrombophilic defects.

The effect of the 4G/5G polymorphism on prognosis in patients with acute severe diseases

Several reports suggest that 4G/4G patients might have a genetic susceptibility to high PAI-1 responses after exposure to noxious factors. PAI-1 is considered an acute-phase reactant and its release is regulated by various inflammatory factors, including interleukin-1, TNF- α , and TGF- β [47]. These agonists directly affect PAI-1 gene expression [48]. Thus, it is assumed that elevation of PAI-1 levels after stressful events, like severe inflammatory diseases or life-threatening disorders, in patients with the 4G allele is much more pronounced, resulting in impaired fibrinolysis and consequently

in impaired microcirculation. This might account for the increased frequency of sepsis and poor outcome in 4G homozygous children with meningococcal infection [49–51], the poor survival rate with high incidence of multiorgan failure in severely injured 4G/4G patients [52], and the increased risk for ischemic stroke after aneurysmal subarachnoid hemorrhage [33] or cardiac surgery [34] in 4G patients. Moreover, elevated plasma levels of PAI-1 have been associated with an unfavourable outcome in patients with sepsis [53,54]. Finally, some studies have also demonstrated increased levels of PAI-1 in bronchoalveolar lavage fluid obtained from patients with acute lung injury, implying that there is a suppression of the normal profibrinolytic environment in the lung [55,56]. However, in these studies the genotype of patients was not determined.

In summary, the great majority of studies support a significant effect of this polymorphism or its gene product on the outcome of diverse severe inflammatory diseases where the interconnection of coagulation and inflammation is well recognized through direct activation of PAI-1 by several cytokines.

Conclusions

PAI-1 is a characteristic biological example of how complicated and unpredictable could be the influence of the same enzyme on different tissues or organs. Improper study designs, case definitions, and selection of controls, can easily increase bias and lead to misleading findings. On the contrary, large sample sizes, detailed description of the eligibility criteria, random selection of the control group from the population that gave rise to the cases, a test for the HWE and the proper adjustment for the other well-established risk factors in the statistical analysis, along with consideration of the underlying pathophysiological mechanisms, may facilitate the reliable detection of those group of patients whose prognosis and treatment can be modified according to this genetic variant.

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