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Relationship between PAI1 promoter 4G/5g polymorphism and stroke

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Abstract

PAI-1 has become recognized as a central molecule linking pathogenesis and progression of thrombotic vascular events, including stroke. Clinical and experimental studies show that PAI-1 deficiencies cause accelerated fibrinolysis and bleeding, whereas elevated PAI-1 plasma levels are associated with vascular thrombosis. Raised PAI1 plasma levels are related to a 1-bp guanine deletion/insertion (4G/5G) polymorphism in the promoter of the PAI1 gene. The 4G allele is associated with higher plasma PAI1 transcription and activity. In the current study, the association of higher PAI-1 plasma levels and the prevalence of the 4G/5G polymorphism in the PAI-1 gene promoter region in young patients with stroke were explored. Significantly, higher PAI-1 levels were observed in patients when compared to controls (p=002). The 4G/5G polymorphisms were significantly associated with increased PAI-1 levels, with the variant homozygous 4G/4G corresponding to mean values in patients versus controls.

INTRODUCTION

The SERPINE1 gene encodes endothelial plasminogen activator inhibitor-1 (PAI1), a member of the serine protease inhibitor family that inhibits the tissue-type plasminogen activator and urokinase-type plasminogen activator (PLAU) (1). PLAT and PLAU proteolytically activate plasminogen (PLG) into plasmin, which breaks down fibrin clots (2). Thus, SERPINE1 negatively regulates fibrinolysis and impairs the dissolution of clots. Alterations in thrombosis and fibrinolysis comprise important parts of stroke pathophysiology (3). A key step in the fibrinolytic process includes the tissue-type plasminogen activator (tPA)-mediated conversion of the proenzyme plasminogen into the active protease plasmin which, in turn, degrades the fibrin structure of intravascular thrombi (4). Inhibition of the fibrinolytic system may occur at the level of plasminogen activation, mainly by a direct inhibition of tPA by plasminogen activator inhibitor 1 (PAI-1) or indirectly by thrombin-activatable fibrinolysis inhibitor and, at the level of plasmin, by α_2-antiplasmin (5). The roles of these three inhibitors are complementary in thrombolysis. PAI-1 has become recognized as a central molecule linking pathogenesis and progression of thrombotic vascular events, including stroke (6). As a main endogenous inhibitor of tPA, PAI-1 might be related to reperfusion efficacy and hemorrhagic risk of tPA thrombolytic therapy (7). The PAI-1 promoter contains a common −675 4G/5G polymorphism that may affect both basal and inducible PAI-1 expression; however, clinical studies have shown divergent results (8). A few studies have reported that individuals having 4G allele in the homozygote condition have increased PAI-1 levels and heterozygotes (4G/5G) have intermediate PAI-1 levels, whereas those homozygotes for the 5G allele have decreased PAI-1 levels (9). The association of 4G/5G polymorphism and PAI-1 levels is also well described in patients with diabetes mellitus and myocardium infarction as well as in healthy participants. Several studies have represented the effect of 4G/5G polymorphisms on the risk of developing stroke, but the results remain controversial (10). In the present study, the association of higher PAI-1 plasma levels and the prevalence of the 4G/5G polymorphism in the PAI-1 gene promoter region were explored in young stroke patients.

METHODS AND MATERIALS

The study participants comprised adults with an average age of 45 years and included 90 patients presenting with acute stroke who were clinically and radiologically diagnosed. The control group was comprised of 90 healthy participants aged (+3 years) and gender-matched with the patients while having no relationship, but c coming from the same

demographic area. Samples were obtained from each participant after at least 12 hours of fasting. Plasma was separated as soon as possible by centrifugation for 15 minutes at 3500 g and stored at 80 °C for the pending analyses. Genomic DNA was extracted from peripheral blood leukocytes using salting-out methods. Plasma PAI-1 levels were evaluated for patients (post-acute phase) as well as controls. The minimum detectable dose of PAI-1 is typically <200 pg/mL. The genotyping for 4G/5G polymorphism was performed RFLP-PCR: The primers used in PCR were: forward 5′-CAC AGA GAG AGT CTG GCC ACGT-3′ and reverse 5′-CCA ACA GAG GAC TCTTGG TCT-39CCACG-3′. Because (-675) 4G/5G polymorphism is based on the insertion-deletion of a G allele in the PAI-1 promoter, the product of the PCR was 98 bp for the 4G allele and 99 bp for the 5G allele. FastDigest Bs lI (Fermentas ®, Thermo Scientific, Waltham, MA, USA) was used to digest the amplification product. Afterwards, digested fragments were analyzed by electrophoresis on 4% polyacrylamide gel. The PAI-1 (-675) 5G allele showed a 77-bp fragment, and the PAI-1 (-675) 4G allele showed a 98-bp fragment.

RESULTS

Significantly, higher PAI-1 levels were observed in patients when compared to controls (p=002). A significantly higher number of patients had high PAI-1 levels than controls; the risk for stroke was increased to almost 6-fold. Genotyping for 5G/4G polymorphism showed prevalence rates of 37 (41%) and 25 (27%) for the 4G/4Ggenotype in the patient and control populations, respectively, which are significantly different (p=0.021). The allelic frequency of the 4G allele was 119 and 96 in the patient and control populations, respectively, which is a significant difference (p=0.018). These results show that 4G allele has a highly significant association with patients with stroke. The 4G/5G polymorphisms were significantly associated with increased PAI-1 levels with the variant homozygous 4G/4G corresponding to mean values in patients versus controls (Table 1).

Table 1. Genotype and Allele Frequency

4G/5G Genotype frequency	Case	Control	Significance
5G/5G	8 (9%)	19 (21%)	0.084
4G/4G	37 (41%)	25 (27%)	0.021
4G/5G	45 (50%)	46 (52%)	0.147
4G/4G Allele frequency			
5G	61	84	0.074
4G	119	96	0.018

DISCUSSION

PAI-1 has become recognized as a central molecule linking the pathogenesis and progression of thrombotic vascular events, including stroke. Clinical and experimental studies have shown that PAI-1 deficiencies cause accelerated fibrinolysis and bleeding, whereas elevated PAI-1 plasma levels are associated with vascular thrombosis (11). Dawson et al. (1993) and Eriksson et al. (1995) demonstrated that raised PAI1 plasma levels are related to a 1-bp guanine deletion/insertion (4G/5G) polymorphism in the promoter of the PAI1 gene. The 4G allele is associated with higher plasma PAI1 transcription and activity (12). Although both alleles bind a transcriptional activator, the 5G allele also binds a repressor protein to an overlapping binding site (13). In the absence of a bound repressor, the 4G allele is associated with an increased basal level of PAI1 transcription. Eriksson et al. found that the prevalence of the 4G allele was significantly higher in patients with myocardial infarction before the age of 45 (allele frequency of 0.63) than in population-based controls (14). An in vitro study by Festa et al. found that the 4G allele expresses almost 6 times more messenger RNA than the 5G allele. Margaglione et al. investigated the relationship between the PAI1 4G/5G polymorphism in 1,179 healthy employees and the occurrence of coronary artery disease in their first-degree relatives (15). The group with a first-degree relative who had suffered from a coronary ischemic episode had a higher number of homozygotes for the 4G allele compared with subjects without such a family history (odds ratio = 1.62) (16). The frequency of the 4G allele was abnormally high in individuals with a family history who also had higher body mass indexes and total cholesterol levels (17). A recent meta-analysis suggested that the highly expressed PAI-1 4G allele may have a protective effect in ischemic stroke, whereas the same allele may be associated with an increased risk of myocardial infarction (18). While lending further support for a neuroprotective role of PAI-1 in the central nervous system, these findings clearly point to a more complex role for tPA and PAI-1 in stroke pathophysiology compared with other thrombotic diseases, thus making the design of PAI-1-targeted treatments more challenging (19-20). The current study also found a significant association between 4G alleles and plasma PAI1 levels and the risk of heart attack. To overcome the limitations of

the study, similar designs must be carried out in large multicentric populations, taking into consideration the survival bias that will account for the genetic heterogeneity of the Indian population.

REFERENCE

1. Dawson SJ, Wiman B, Hamsten A, et al. The two allele sequences of a common polymorphism in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene respond differently to interleukin-1 in HepG2 cells. JBiolChem. 1993;268(15):10739-10745.
2. Klinger KW, Winqvist R, Riccio A, et al. Plasminogen activator inhibitor type 1 gene is located at region q21.3-q22 of chromosome 7 and genetically linked with cystic fibrosis. Proc Natl Acad Sci U S A. 1987;84(23):8548-8552.
3. Klinger KW, Winqvist R, Riccio A, et al. Plasminogen activator inhibitor type 1 gene is located at region q21.3-q22 of chromosome 7 and genetically linked with cystic fibrosis. Proc Natl Acad Sci U S A. 1987;84(23):8548-8552.
4. Tsai SJ, Hong CJ, Liou YJ, Yu YW, Chen TJ. Plasminogen activato inhibitor-1 gene is associated with major depression and antidepressant treatment response. Pharmacogenet Genomics 2008;18(10):869-875.
5. Hoekstra T, Geleijnse JM, Kluft C, Giltay EJ, Kok FJ, Schouten EG. 4G/4G genotype of PAI-1 gene is associated with reduced risk of stroke in elderly Stroke. 2003;34:2822–2829.
6. Catto AJ, Carter AM, Stickland M, et al. Plasminogen activator inhibitor-1(PAI-1) 4G/5G promoter polymorphism and levels in subjects with cerebrovascular disease. Thromb Haemost. 1997;77:730 –734.
7. Heijmans BT, Westendorp RG, Knook DL, et al. Angiotensin I-converting enzyme and plasminogen activator inhibitor-1 gene variants: risk of mortality and fatal cardiovascular disease in an elderly population-based cohort. J Am Coll Cardiol. 1999;34:1176 –1183.
8. Roest M, van der Schouw YT, Banga JD, et al. Plasminogen activator inhibitor 4G polymorphism is associated with decreased risk of cerebrovascular mortality in older women. Circulation. 2000;101:67–70.
9. Elbaz A, Cambien F, Amarenco P. Plasminogen activator inhibitor genotype and brain infarction. Circulation. 2001;103:E13–E14. Endler G, Lalouschek W, Exner M, et al. The 4G/4G genotype at nucleotide position -675 in the promotor region of the plasminogen activator inhibitor 1 (PAI-1) gene is less frequent in young patients with minor stroke than in controls. Br J Haematol. 2000;110:469 – 471.
10. Hindorff LA, Schwartz SM, Siscovick DS, et al. The association of PAI-1 promoter 4G/5G insertion/deletion polymorphism with myocardial infarction and stroke in young women. J Cardiovasc Risk. 2002;9:131–137.
11. Petrovic D, Milanez T, Kobal J, et al. Prothrombotic gene polymorphisms and atherothrombotic cerebral infarction. Acta Neurol Scand. 2003;108:109 –113.
12. Eriksson P, Kallin B, 't Hooft FM, et al. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. Proc Natl Acad Sci U S A. 1995;92: 1851–1855.
13. Hoekstra T, Geleijnse JM, Kluft C, Giltay EJ, Kok FJ, Schouten EG. 4G/4G genotype of PAI-1 gene is associated with reduced risk of stroke in elderly Stroke. 2003;34:2822–2829.
14. Catto AJ, Carter AM, Stickland M, et al. Plasminogen activator inhibitor-1(PAI-1) 4G/5G promoter polymorphism and levels in subjects with cerebrovascular disease. Thromb Haemost. 1997;77:730 –734.
15. Heijmans BT, Westendorp RG, Knook DL, et al. Angiotensin I-converting enzyme and plasminogen activator inhibitor-1 gene variants: risk of mortality and fatal cardiovascular disease in an elderly population-based cohort. J Am Coll Cardiol. 1999;34:1176 –1183.
16. Roest M, van der Schouw YT, Banga JD, et al. Plasminogen activator inhibitor 4G polymorphism is associated with decreased risk of cerebrovascular mortality in older women. Circulation. 2000;101:67–70.
17. Elbaz A, Cambien F, Amarenco P. Plasminogen activator inhibitor genotype and brain infarction. Circulation. 2001;103:E13–E14.
18. Endler G, Lalouschek W, Exner M, et al. The 4G/4G genotype at nucleotide position -675 in the promotor region of the plasminogen activator inhibitor 1 (PAI-1) gene is less frequent in young patients with minorstroke than in controls. Br J Haematol. 2000;110:469 – 471.
19. Hindorff LA, Schwartz SM, Siscovick DS, et al. The association of PAI-1 promoter 4G/5G insertion/deletion polymorphism with myocardial infarction and stroke in young women. J Cardiovasc Risk. 2002;9:131–137.
20. Petrovic D, Milanez T, Kobal J, et al. Prothrombotic gene polymorphisms and atherothrombotic cerebral infarction. Acta Neurol Scand. 2003;108:109 –113.