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The Association of the -675 4G/5G and A-844G Polymorphisms of the Plasminogen Activator Inhibitor-1 Gene with a Risk of Ischemic Stroke in Korean Population

Byung-Cheol Lee, Sang-Hyuk Byun, Soon-Il Kim, Ki-Hoon Kang, Se-Young Ahn, Ho-Kyung Doo, Jung-Chul Seo*, Kang-Hyun Leem**, Seong-Ho Cho***, Chad K. Oh*** and Young-Min Ahn

Department of Internal Medicine, College of Oriental Medicine, Kyung Hee University, Seoul, Korea
Department of Acupuncture and Moxibution, College of Oriental Medicine, Kyungsan University, Gumi 730-090, Korea*
College of Oriental Medicine, Semyung University, Jecheon 390-711, Korea**
Department of Pediatrics, Harbor-UCLA Medical Center, UCLA School of Medicine, Torrance, USA***

한국인의 허혈성 뇌경색 발생과 플라스미노겐 활성 억제인자-1(PAI-1) 유전자 다형성과의 관계

이병철, 변상혁, 김순일, 강기훈, 안세영, 두호경, 서정철*, 임강현**, 조성호***, Chad K. Oh***, 안영민

경희대학교 한의과대학 신계내과학 교실
경산대학교 한의과대학 침구학 교실*
세명대학교 한의과대학**
미국 UCLA대학교 의과대학 Harbor-UCLA 의료원 소아과학 교실***

연구배경 : 플라스미노겐 활성 억제인자-1 (plasminogen activator inhibitor-1; PAI-1)은 허혈성 뇌경색의 발생의 원인이 되는 섬유소 용해작용의 저하를 매개하는 인자로서, PAI-1의 작용이 촉진되면 섬유소 용해기능이 저하되어 관상동맥 및 뇌혈관질환의 발생을 증가시키게 된다. PAI-1 유전자의 촉진자(promotor) 영역에는 -675 4G/5G (4G/5G)와 A -844G (A/G)의 두 개의 유전자 다형성이 존재하며, 이는 PAI-1의 유전자 전사과정에 영향을 미쳐 혈청 PAI-1의 농도를 증가시키고 결과적으로 허혈성 뇌경색의 발생확률을 높이는 작용을 하게 된다.

연구방법 : 허혈성 뇌경색으로 진단 받은 167명의 환자와 173명의 건강인의 말초혈액에서 DNA를 분리한 후 PAI-1의 4G/5G와 A/G 유전자 다형성에 대한 연쇄중합반응 및 제한효소 절편길이 다형성 (polymerase chain reaction-restriction fragment length polymorphism; PCR-RFLP) 방법을 이용하여 허혈성 뇌경색 발생과 유전자 다형성과의 관계를 비교 분석하였다.

결과 : 허혈성 뇌경색 환자에서의 4G/4G의 유전자형의 빈도는 15.0%으로 정상 대조군의 33.5%에 비해 현저하게 낮게 나타났다 ($P < 0.0001$). 각각의 유전자형과 허혈성 뇌경색의 발생 위험도 (odd ratio ; OR)와의 관계를 분석했을 때 4G/4G 유전자형을 가질 경우 위험도는 0.35배로 현저하게 낮아졌으며, ($P < 0.0001$), 5G/5G 유전자형을 가질 경우 위험도는 4.49배로 현저하게 높아졌다 ($P < 0.0001$). 그러나, A/G 유전자 다형성과 허혈성 뇌경색의 발생과는 유의한 연관성을 발견하지 못하였다.

결론 : 이상의 결과로 볼 때 PAI-1 유전자의 4G/4G 유전자형은 허혈성 뇌경색의 발생 비율을 감소시키는 작용을 하는 것으로 여겨진다.

Key Words: 플라스미노겐 활성 억제인자-1, 유전자 다형성, 허혈성 뇌경색

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· 교신저자 : 안영민, 서울시 동대문구 회기동 1 경희의료원
부속한방병원 한방 6내과
(Tel. 02-958-9097 Fax. 02-958-9158
E-mail : omdan@hanmail.net)
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I. Introduction

Ischemic stroke is a common cause of morbidity and mortality. Fibrinolytic activity is an important predictor for ischemic events in atherosclerotic patients[1]. The decreased fibrinolysis is mainly due to elevated plasminogen activator inhibitor (PAI)-1 which is the major inhibitor of both tissue-type and urokinase-type plasminogen[2].

PAI-1 is a 50-kD glycoprotein that belongs to the serine protease inhibitor superfamily[3,4]. Elevated plasma PAI-1 activity is observed in patients with ischemic stroke or coronary heart disease[5]. The human PAI-1 gene has eight polymorphisms that have been described to date. Two polymorphisms in the promoter region of the PAI-1 gene, 675 4G/5G (4G/5G) and A-844G (A/G), are of interest because of their association with altered PAI-1 transcription[6]. The 4G/5G polymorphism is associated with a risk for atherosclerotic diseases such as stroke and myocardial infarction[7,8,9]. However, whether the 4G allele increases or decreases the risk of atherosclerotic diseases is controversial. Contrasting the previous studies, recent studies suggest that the 4G allele is not a risk factor but a beneficial survival factor[10,11,12]. However, the mechanism is still unknown. The A/G polymorphism is located in a consensus sequence-binding site of Ets nuclear protein[13]. Recently, Morange et al.[14] reported the association between the A allele and a higher risk for vascular thrombosis. However, the association of the A/G polymorphism with ischemic stroke is unknown.

In this study, we evaluated whether the 4G/5G and A/G polymorphisms of the PAI-1 gene is associated with a risk of ischemic stroke in relatively genetically homogeneous Korean population.

II. Materials and Methods

1. Patients and control subjects

Participating patients were consisted of 167 ischemic stroke patients diagnosed between January 2001 and December 2002 at the Kyung Hee University Medical Center in Seoul, Korea. Control subjects were comprised of 173 healthy blood donors attending the center for regular health check-ups (Table 1). For this study, ischemic stroke was defined as rapidly developing clinical symptoms or signs of focal and global loss of brain function with symptoms lasting more than 24 hours. The symptoms and signs are consecutively accompanied by corresponding focal or diffuse density changes detected by brain computed tomography (CT) or magnetic resonance imaging (MRI). Ischemic stroke patients with an apparent cause other than that of vascular origin were excluded. Patients with cerebral hemorrhage, cerebral venous thrombosis and brain tumor were also excluded. The following baseline characteristics and vascular risk factor were recorded; age, gender, hypertension and non-insulin independent diabetic mellitus (NIDDM). Control subjects were free of hypertension, NIDDM, ischemic stroke, ischemic heart disease, transient ischemic attack, prior stroke or disease concerning vascular risk factor. This study was approved by the ethics review committee of the Medical Research Institute at the Kyung Hee University Medical Center in Seoul, Korea.

2. DNA Isolation and Genotyping

DNA was isolated from venous blood using a genomic DNA purification kit (Nucleospin®, Düren, Germany). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)-based genotyping of the 4G/5G and A/G polymorphisms

were performed as described[6,15]. Briefly, PCR was carried out in each sample contained genomic DNA from patients or controls with the following primers: the 4G/5G polymorphism, 5' - TCCAACCTCAGCCAGACAAG - 3' and 5' - TGATA CACGGCTGACTCACC - 3'; the A/G polymorphism, 5' - CAGGCTCCCACTGATTCTAC - 3' and 5' - GAGGGCTCTCTTGTGTCAAC - 3'. The amplification products for the 4G/5G and A/G polymorphisms were digested with *Dra*III and *Xho*I (New England Biolabs, Beverly, USA), respectively at 37oC. DraIII digested the amplified products from the 4G allele into 2 fragments of 71 and 19 bp, and products amplified from gene with the 5G allele remain undigested. XhoI digested the amplified products from the G allele into 2 fragments of 364 bp and 146 bp, and products amplified from gene with the A allele remain undigested. The accuracy of PCR-RFLP was confirmed by three times of analysis and direct sequencing in several randomly selected samples.

3. Statistical Analysis

The differences of observed genotypes and alleles between ischemic stroke patients and controls were compared by chi-square (x^2) test. Two-sided P values of < 0.05 was considered significant. Odds ratio (OR) and 95% confidence intervals (CI) were calculated as estimates of the relative risks. The GraphPad PRISM statistical package (ver 2.00, Graphpad software inc., San Diego, USA) was used.

III. Results

To determine whether the 4G/5G polymorphism is associated with ischemic stroke, a total of 340 participants (173 controls, 167 ischemic stroke patients) were genotyped for the 4G/5G polymorphism. The distribution of 4G/4G genotype (15%) in the patients with ischemic stroke was significantly different from the distributions seen in the healthy controls (33.5%). Decreased risk of ischemic stroke was associated with the 4G/4G genotype (OR = 0.35, 95% CI 0.21-0.59, $P < 0.0001$) and increased risk of ischemic stroke was associated with the 5G/5G genotype (OR = 4.49, 95% CI 2.60-7.73, $P < 0.0001$) (Table 2). The prevalence of the 4G allele in the patient group was also significantly lower than that in the healthy control (0.38 vs 0.60, $P < 0.0001$), and the 5G allele frequency was higher (0.62 vs 0.40, $P < 0.0001$). To determine the related-factors of ischemic stroke, the association between the 4G/5G polymorphism and age of onset/gender was assessed. In both females and males, 4G/4G genotype was decreased in

Table 1. Clinical Characteristics of Ischemic Stroke Patients and Controls.

Characteristics	Control	Stroke
N	173	167
Age, y	62.0 ± 9.1	63.1 ± 11.0
Male gender, n (%)	83 (48.0)	90 (54.0)
Stroke type, n (%)		
Atherosclerotic	-	85 (50.9)
Lacunar	-	47 (28.1)
Cardioembolic	-	23 (13.8)
Others	-	12 (7.2)
History of NIDDM, n (%)	-	66 (39.5)
History of hypertension, n (%)	-	90 (53.9)

N = number; NIDDM = non-insulin independent diabetic mellitus.

patients (OR = 0.33 and OR = 0.38, respectively) and 5G/5G genotypes was increased (OR = 2.22 and OR = 10.74, respectively). However, there was no significant difference in accordance with age of onset.

The association of the A/G polymorphism with a risk of ischemic stroke was also evaluated by genotyping. No significant differences were detected in the allelic and genotype distributions between ischemic stroke patients and healthy controls (Table 3).

IV. Discussion

In this study, we presented the association of the 4G/5G and A/G polymorphisms with a risk of ischemic stroke in a relatively genetically homogeneous Korean population. The distributions of the 4G/5G and A/G alleles in control subjects are different from that in Caucasian population, although the allelic frequency of the polymorphisms are consistent with those reported in other Korea study[16]. For example, 4G allele frequency in Korean population is higher than that in Caucasian population, and the A allele was more common than G allele in Caucasian group[14,17,18], whereas the G allele was more frequent than the A allele in our controls. We speculate that the difference could be due to the racial difference, although more definitive explanation could come from large population-based studies.

The 4G/4G genotype was associated with reduced risk for ischemic stroke. This result confirms the previous finding indicating a protective effect of the 4G/4G genotype in ischemic stroke[10,12]. In the

Table 2. Distribution of the 4G/5G Polymorphisms in Ischemic Stroke Patients and Controls.

	Control (n = 173)	Stroke (n = 167)	OR (95% CI)	*P* value*
4G/5G genotypes				
4G/4G	58 (0.34)	25 (0.15)	0.35 (0.21-0.59)	<0.0001
4G/5G	93 (0.54)	76 (0.46)	0.72 (0.47-1.10)	0.1283
5G/5G	22 (0.13)	66 (0.39)	4.49 (2.60-7.73)	<0.0001
4G/5G alleles				
4G	209 (0.60)	126 (0.38)		<0.0001
5G	137 (0.40)	208 (0.62)		

OR = odds ratio; CI = confidence intervals.
*x^2 test; stroke patients versus control subjects in each analysis.

Table 3. Distribution of the A/G polymorphisms in ischemic stroke patients and controls.

	Control (n = 173)	Stroke (n = 167)	OR (95% CI)	
A/G genotypes				
AA	25 (0.15)	24 (0.14)	1.01 (0.55-1.84)	0.9833
AG	99 (0.57)	85 (0.51)	0.77 (0.51-1.19)	0.2418
GG	49 (0.28)	58 (0.35)	1.35 (0.85-2.13)	0.2035
A/G alleles				
A	149 (0.43)	133 (0.40)		0.1342
G	197 (0.57)	201 (0.60)		

OR = odds ratio; CI = confidence intervals.
*x^2 test; stroke patients versus control subjects in each analysis.

previous reports, the association between the 4G/4G genotype and a lower risk of ischemic stroke was limited to minor stroke or transient ischemic attack (TIA) and young women group. However, our study indicates that the protective effect of the 4G polymorphism was present in all age and gender group. It is known that the 4G allele is associated with increased basal level of PAI-1 gene transcription[19]. Therefore, the 4G/4G genotype was thought to be associated with increased plasma PAI-1 level which may inhibit fibrinolysis and increase the risk of atherosclerosis and thrombosis. However, recent data and our study indicate that the 4G/4G genotype may reduce the risk of ischemic stroke. One of the possible explanations is that increased PAI-1 activity in the atherosclerotic plaque may inhibit plasminogen activators, which in turn protects the fibrous cap against degradation by decreasing matrix metalloproteinase activity and subsequently against rupture[20].

The association of the A/G polymorphism with a risk of ischemic stroke was also evaluated by genotyping. No significant differences in the allelic and genotypic distributions were detected between ischemic stroke patients and healthy controls. Our results indicate that the A allele or A/A genotype is not a risk factor for ischemic stroke in the Korean population. Recently, Haselbauer et al.[18] reported that the A/G polymorphism is not an independent risk factor of coronary artery disease. Grubic et al.[17] reported that there was a lack of association between the A/G polymorphism and plasma PAI-1 level even though functional importance of the A/G polymorphism in regulating the expression of PAI-1 gene was demonstrated in specific binding study of a nuclear protein. Taken together, the A allele is associated with a higher risk of vascular thrombosis in factor V Laiden carriers[14], but not with atherosclerotic diseases such as myocardial infarction and ischemic stroke. In summary, our findings suggest that the 4G/4G, but not A/A, genotype in the PAI-1 gene is associated with decreased risk of ischemic stroke.

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