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Association of the Plasminogen Activator Inhibitor-1 (PAI-1) Gene -675 4G/5G and -844 A/G Promoter Polymorphism with Risk of Keloid in a Chinese Han Population

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: A keloid is pathological scar caused by aberrant response to skin injuries, characterized by excessive accumulation of histological extracellular matrix, and occurs in genetically susceptible individuals. Plasminogen activator inhibitor-1 (PAI-1) has been implicated in the pathogenesis of keloid. We investigated the association between PAI-1 polymorphisms and plasma PAI-1 level with keloid risk.

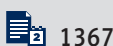
Material/Methods: A total of 242 Chinese keloid patients and 207 controls were enrolled in this study. Polymerase chain reaction-restriction technique was used to determine PAI-1 promoter polymorphism (-675 4G/5G and -844 A/G) distribution. Plasma PAI-1 levels were detected using enzyme-linked immunosorbent assay (ELISA).

Results: There was a statistically significant difference in the distribution of PAI-1 -675 4G/5G polymorphism between keloid patients and healthy controls. 4G/4G carriers were more likely to develop keloid. In contrast, the -844 A/G polymorphism distribution did not vary significantly between keloid patients and controls. The keloid patients group had a significantly higher plasma PAI-1 level than the control group. In the -675 4G/4G carrier population, the plasma PAI-1 levels were significant higher in keloid patients compared with controls.

Conclusions: Our study provides evidence that PAI-1 promoter polymorphism -675 4G/5G and plasma PAI-1 level are associated with keloid risk. PAI-1 -675 4G/5G polymorphism may be an important hereditary factor responsible for keloid development in the Chinese Han population.

MeSH Keywords: Keloid • Plasminogen Activator Inhibitor 1 • Polymorphism, Single Nucleotide

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Background

Keloid is a raised skin scar that is due to an aberrant response to skin injuries and is characterized by an excessive accumulation of extracellular matrix [1]. The prevalence of keloid varies among different populations. The incidence can be as high as 16% in Africans. Compared to Caucasians, Asians also have a higher prevalence rate [2,3]. Keloid is a complex condition with environmental and genetic risk-contributing factors. However, genetic factors play a very important role in the development and progression of keloid [4–6]. Several genetic polymorphisms have been identified to be associated with keloid risk [7–9].

Plasminogen activator inhibitor-1 (PAI-1), also known as endothelial plasminogen activator inhibitor, is the principal physiological inhibitor of the plasminogen activator/plasmin protease system [10]. Increased PAI-1 activity has been noted to be a feature of fibrosis. Accumulating evidence shows there is a direct correlation between the extent of collagen accumulation in the process of injury repair and genetically determined level of PAI-1. In keloid tissue, PAI was dramatically increased. PAI-1 may contribute to keloid pathogenesis through increasing accumulation of collagen synthesized by keloid fibroblasts [11,12].

Several polymorphisms within the PAI-1 gene influence PAI-1 levels and are associated with incidence of several diseases. Among these polymorphisms, the -675 4G/5G insertion-deletion mutation and the -844G/A single-nucleotide polymorphism (SNP) have received much attention [13–16]. So far, there is no study investigating the association between PAI-1 polymorphisms and the risk for KS. Based on this knowledge, we wondered whether these PAI-1 polymorphisms might be associated with keloid pathogenesis. The aim of the present study was to investigate the impact of -675 4G/5G and -844G/A polymorphisms in PAI-1 on susceptibility of keloid and serum PAI-1 level in a Chinese Han population.

Material and Methods

Subjects

We enrolled 241 patients with keloid from our hospital. The diagnosis of all cases was made by 2 independent physicians. The controls group consisted of 207 normal healthy individuals without a diagnosis of keloid, family history of keloid, or autoimmune disorders. The study was approved by our hospital's ethics committees and written informed consent was obtained from each subject.

Methods

Blood specimens (10 ml) were collected from each individual. The genomic DNA was isolated with standard phenol-chloroform

method. Plasma level of PAI-1 was measured by use of an AssayMax Human Plasminogen Activator Inhibitor-1 ELISA kit (AssayPro, St. Charles, MO, USA).

PAI-1 (-675) 4G/5G polymorphism and -844G/A polymorphisms were determined using a standard polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay.

PAI-1 (-675) 4G/5G

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'. Since (-675) 4G/5G polymorphism is a polymorphism based on the insertion-deletion of a G allele in PAI-1 promoter, the product of the PCR was 98 bp for the 4G allele and 99 bp for the 5G allele. FastDigest BslI (Fermentas®, Thermo Scientific, Waltham, MA, USA) was used to digest the amplification product. Afterwards, digested fragments were analyzed by electrophoresis on 4% polyacrylamide gel. PAI-1 (-675) 5G allele showed 77-bp fragment, and PAI-1 (-675) 4G allele showed 98-bp fragment.

-844G/A

The -844G/A polymorphism was determined by PCR-RFLP, using the following primers: forward, 5'-CAGGCTCCCACTGATTCTAC-3'; and reverse, 5'-GAGGGCTCTTGTGTCAAC-3'. Amplified fragments were digested with FastDigest XhoI (Fermentas®, Thermo Scientific, Waltham, MA, USA) and analyzed by 3% agarose gel electrophoresis. We obtained 314+146 bp fragments for -844G allele and 510-bp fragment for -844A allele.

Statistical analysis

The *t* test was used to compare the clinical features between the keloid patients and controls. Chi-square tests were used to analyze the genotype frequency and demographic distributions between cases and controls and to evaluate the Hardy-Weinberg equilibrium. Logistic regression analysis was used to determine the odds ratios (ORs) and 95% confidential intervals (CIs) for genotypes and alleles in keloid patients and controls. All statistical analyses were performed with SPSS version 17.0 software (SPSS Company, Chicago, Illinois, USA). $P < 0.05$ was considered statistically significant.

Results

The clinical features in the keloid and control groups are presented in Table 1. There were no significant differences between keloid and control group in mean age, sex, or BMI ($P > 0.05$). KS patients had significantly higher plasma PAI-1 level than controls ($P < 0.001$). The distribution of PAI-1-844 A/G and -675

Table 1. The clinical characteristics of all subjects.

	Keloid group (n=242)	Control group (n=207)	P value
Sex (F: M)	105: 137	98: 109	0.401
Age	22.1±2.36	21.3±2.14	0.244
BMI	23.6±1.81	22.9±1.95	0.556
Race or ethnicity	Chinese Han	Chinese Han	1
Plasma PAI-1 level	23.02±3.98	20.25±2.45	<0.001

Table 2. The genotype and the allele frequencies of PAI-1 gene polymorphism in subjects and relative risk for keloid compared with controls.

		Keloid group n (%)	Control group n (%)	OR	95% CI	P value
(-675) 4G/5G	4G/4G	94 (38.8)	58 (28.0)	1.549	1.017–2.358	0.041
	5G/4G	113 (46.7)	108 (52.2)	1 (reference value)		
	5G/5G	35 (14.5)	41 (19.8)	0.816	0.484–1.376	0.445
	5G	183 (37.8)	190 (45.9)	1 (reference value)		
	4G	301 (62.2)	224 (54.1)	1.395	1.069–1.822	0.014
-844A/G	GG	63 (26.0)	68 (32.9)	0.746	0.484–1.150	0.184
	AG	123 (50.8)	99 (47.8)	1 (reference value)		
	AA	56 (23.1)	40 (19.3)	1.127	0.694–1.829	0.629
	G	249 (51.4)	186 (56.8)	1 (reference value)		
	A	235 (48.6)	1794 (3.2)	1.239	0.952–1.613	0.111

4G/5G polymorphisms were found to be in Hardy-Weinberg equilibrium for Keloid patients and controls (all $P>0.05$)

The genotype distribution of PAI-1 -844 A/G and -675 4G/5G polymorphisms was not significantly different between the KS and controls ($P>0.05$) (Table 2). However, the distribution of 4G/5G genotypes was significantly different between the keloid group and the controls. The frequencies of 4G/4G, 4G/5G, and 5G/5G genotypes were 39%, 47%, and 14%, respectively, in the KS patients. The prevalence of the -675 4G/4G genotype was markedly higher in keloid patients than in controls (39% vs. 28%, $P=0.041$). With 4G/5G of as reference, the statistic analysis showed the 4G/4G genotype carriers had a higher chance of having KS (OR=1.549, 95% CI 1.017–2.358). Similarly, the PAI-1 gene 4G allele was significantly higher in keloid patients compared with controls (62.2% vs. 54.1%, $P=0.014$). These differences represented an increased risk of keloid as evidenced by the OR of 1.395 (95% CI 1.069–1.822).

We analyzed the influence of the PAI-1 -844 A/G and -675 4G/5G polymorphisms on the serum PAI-1 levels (Table 3). Keloid

patients had significantly higher PAI-1 levels than the control group (23.00±3.98) vs. (20.25±2.45) ng/ml, $P<0.0001$) (Table 1). As shown in Table 3, the plasma PAI-1 level was significantly higher in patients with 4G/4G genotype than those without 4G/4G genotypes. Keloid patients with 4G/4G genotype had remarkably higher PAI-1 levels than those subjects with 4G/4G genotype in the control group ($P<0.001$). However, when comparing keloid patients and controls with other genotypes, we did not find any other genotypes that could affect the plasma PAI-1 level.

Discussion

In the present study, we investigated the effect of 2 PAI-1 gene polymorphisms on risk of KS in a Chinese Han population. PAI-1 has been shown to be crucial to the plasminogen activator/plasmin protease system. It plays an important role in a wide variety of physiologic and pathologic processes, including fibrosis, fibrinolysis, wound healing, and cancer metastasis. In normal skin, PAI-1 was present at low levels, whereas in keloid tissue PAI was dramatically increased. Emerging evidence

Table 3. The plasma PAI-1 levels of keloid patients and controls in each genotype of PAI-1 polymorphism.

		Keloid group (ng/ml)	Control group (ng/ml)	P value
(-675)4G/5G	4G/4G	26.34±3.52	22.14±3.36	<0.001
	5G/4G	20.71±2.41	21.18±2.99	0.201
	5G/5G	21.86±3.07	23.34±3.78	0.067
-844A/G	GG	21.25±2.91	20.44±2.43	0.086
	AG	23.98±4.09	22.99±3.51	0.059
	AA	23.09±4.12	21.55±3.39	0.056

demonstrates that the relationship between PAI-1 and collagen accumulation is an important mechanism in the development of keloid [11,12].

There are many reports indicating that several common polymorphisms of the PAI-1 gene are risk factors for various diseases related to the elevated serum levels of PAI-1 [13,17]. However, to the best of our knowledge, this is the first study to investigate the association between PAI-1 polymorphisms with the risk of keloid. We demonstrated that the PAI-1 -675 4G/5G polymorphism, but not -844 A/G polymorphism, was associated with keloid incidence. Our findings show that PAI-1 -675 4G/4G carriers had a higher risk of developing keloid. Several studies reported that the PAI-1 -844 A/G polymorphism may contribute to the risk of some diseases [18,19]. However, in the present study, no evidence was found of a relationship between this variant and the risk of keloid.

In the present study, keloid subjects had a significantly higher mean plasma PAI-1 level than controls. Previous studies showed that the -675 4G/5G promoter polymorphism of PAI-1 may modulate PAI-1 transcription. The 4G allele insertion/deletion promoter polymorphism has been associated with elevated

plasma levels of PAI-1 [13,17]. Our study demonstrated that the patients with 4G/4G genotype had a significantly higher plasma PAI-1 level compared with 4G/5G genotype and 5G/5G genotype. Keloid patients with 4G/4G genotype had remarkably higher plasma PAI-1 level than those subjects in the control group. Collectively, our findings provide evidence that PAI-1 plays an important role in the development of KS. The -675 4G/5G gene polymorphisms of PAI-1 and the circulating PAI-1 are associated with risk of keloid.

Conclusions

In conclusion, our study presents evidence of an association of PAI-1 -675 4G/5G polymorphism with keloid risk and plasma PAI-1 levels in a Chinese Han population. Since this was a single-center study and the number of subjects was relatively small, it is necessary to perform larger studies investigating the genetics of keloid.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References:

- Lee JY, Yang CC, Chao SC, Wong TW: Histopathological differential diagnosis of keloid and hypertrophic scar. *Am J Dermatopathol*, 2004; 26: 379–84
- Marners AG, Krieg T: Keloids – clinical diagnosis, pathogenesis, and treatment options. *J Dtsch Dermatol Ges*, 2004; 2: 905–13
- Bayat A, Arscott G, Ollier WE et al: Keloid disease: clinical relevance of single versus multiple site scars. *Br J Plast Surg*, 2005; 58: 28–37
- Shih B, Bayat A: Genetics of keloid scarring. *Arch Dermatol Res*, 2010; 302: 319–39
- Zhu F, Wu B, Li P et al: Association study confirmed susceptibility loci with keloid in the Chinese Han population. *PLoS One*, 2013; 8(5): e62377
- Nakashima M, Chung S, Takahashi A et al: A genome-wide association study identifies four susceptibility loci for keloid in the Japanese population. *Nat Genet*, 2010; 42: 768–71
- Ogawa R, Watanabe A, Naing BT et al: Associations between Keloid Severity and Single-Nucleotide Polymorphisms: Importance of rs8032158 as a Biomarker of Keloid Severity. *J Invest Dermatol*, 2014; 134(7): 2041–43
- Yu D, Shang Y, Luo S, Hao L: The TaqI gene polymorphisms of VDR and the circulating 1,25-dihydroxyvitamin D levels confer the risk for the keloid scarring in Chinese cohorts. *Cell Physiol Biochem*, 2013; 32(1): 39–45
- Wu Y, Wang B, Li YH et al: Meta-analysis demonstrates association between Arg72Pro polymorphism in the P53 gene and susceptibility to keloids in the Chinese population. *Genet Mol Res*, 2012; 11(2): 1701–11
- Cale JM, Lawrence DA: Structure-function relationships of plasminogen activator inhibitor-1 and its potential as a therapeutic agent. *Curr Drug Targets*, 2007; 8: 971–81
- Tuan TL, Hwu P, Ho W et al: Adenoviral overexpression and small interfering RNA suppression demonstrate that plasminogen activator inhibitor-1 produces elevated collagen accumulation in normal and keloid fibroblasts. *Am J Pathol*, 2008; 173(5): 1311–25
- Syed F, Bagabir RA, Paus R, Bayat A: *Ex vivo* evaluation of antifibrotic compounds in skin scarring: EGCG and silencing of PAI-1 independently inhibit growth and induce keloid shrinkage. *Lab Invest Aug*, 2013; 93(8): 946–60

13. Lin S, Huiya Z, Bo L, Wei W, Yongmei G: The plasminogen activator inhibitor-1 (PAI-1) gene -844 A/G and -675 4G/5G promoter polymorphism significantly influences plasma PAI-1 levels in women with polycystic ovary syndrome. *Endocrine Dec*, 2009; 36(3): 503–9
14. Magdoud K, Herbepin VG, Touraine R et al: Plasminogen activator inhibitor 1 4G/5G and -844G/A variants in idiopathic recurrent pregnancy loss. *Am J Reprod Immunol*, 2013; 70(3): 246–52
15. Han SR, Kim CJ, Lee BC: Impact of the -675 4G/5G polymorphism of the plasminogen activator inhibitor-1 gene on childhood IgA nephropathy. *Exp Ther Med*, 2012; 3(4): 703–6
16. Xu X, Xie Y, Lin Y et al: PAI-1 promoter 4G/5G polymorphism (rs1799768) contributes to tumor susceptibility: Evidence from meta-analysis. *Exp Ther Med*, 2012; 4(6): 1127–33
17. Sogutlu Sari E, Yazici A, Eser B et al: The prevalence of 4G/5G polymorphism of plasminogen activator inhibitor-1 (PAI-1) gene in central serous chorioretinopathy and its association with plasma PAI-1 levels. *Cutan Ocul Toxicol*, 2014; 1–5 [Epub ahead of print]
18. De la Cruz-Mosso U, Muñoz-Valle JF, Salgado-Goytia L et al: Relationship of metabolic syndrome and its components with -844 G/A and HindIII C/G PAI-1 gene polymorphisms in Mexican children. *BMC Pediatr*, 2012; 12: 41
19. Kim H, Cho C, Cho Y et al: Significant associations of PAI-1 genetic polymorphisms with osteonecrosis of the femoral head. *BMC Musculoskeletal Disord*, 2011; 12: 160