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Original article

Diabetic retinopathy, PAI-1 4G/5G and −844G/A polymorphisms, and changes in circulating PAI-1 levels in Tunisian type 2 diabetes patients

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Abstract

Aim. – The association of altered plasminogen activator inhibitor (PAI)-1 levels and PAI-1 polymorphisms (4G/5G and −844G/A) with diabetic retinopathy (DR) was investigated in 856 type 2 diabetes (T2D) patients, of whom 383 presented with (DR group), and 473 presented without (DWR group), retinopathy.

Methods. – PAI-1 4G/5G and −844G/A genotyping were done by PCR-RFLP, and PAI-1 levels were measured by ELISA testing.

Results. – The genotype distribution of 4G/5G and −844G/A polymorphisms did not deviate from the Hardy-Weinberg equilibrium model among healthy subjects. Higher frequencies of the 4G/4G genotype, and lower frequencies of the −844A allele, −844G/A and −844A/A genotypes, were seen in DR patients, conferring disease susceptibility and protection, respectively. While PAI-1 levels were significantly elevated in the 4G/4G compared with other PAI-1 genotypes, significant differences in PAI-1 levels between DR and DWR patients were seen in the 4G/−844A, 4G/−844G and 5G/−844A haplotype carriers among DR patients. However, comparable distributions of 4G/5G and −844G/A alleles, genotypes and haplotypes, and similar PAI-1 levels, were seen in the proliferative retinopathy (PR) and non-proliferative retinopathy (NPR) patients, indicating that neither PAI-1 variants nor changes in PAI-1 levels were linked to DR severity. Multivariate analyses identified 4G/−844A and 4G/−844G haplotypes as negatively and positively associated, respectively, with DR, but not with DR severity (PR vs NPR) after adjusting for a number of covariates.

Conclusion. – The present study identifies changes in PAI-1 levels and genetic variations at the PAI-1 locus as risk factors for DR, but not DR severity, that may serve as useful markers of increased DR susceptibility.

Keywords: Diabetes; Retinopathy; Plasminogen activator inhibitor-1; Polymorphism; PCR

Résumé

Rétinopathie diabétique, polymorphismes du gène PAI-1 (4G/5G et −844G/A) et variations des taux du PAI-1 chez des diabétiques de type 2 tunisiens.

Objectif. – L'association des variations du taux de l'inhibiteur de l'activateur du plasminogène (PAI)-1 et des polymorphismes (4G/5G et −844G/A) du gène PAI-1 avec la rétinopathie diabétique (RD) a été étudiée chez 856 patients diabétiques de type 2 (DT2), dont 383 présentaient une rétinopathie diabétique (groupe RD) et 473 qui en étaient indemnes (groupe SRD).

Méthodes. – Le génotypage des génotypes 4G/5G et −844G/A du PAI-1 a été réalisé par PCR-RFLP et les taux de PAI-1 ont été dosés par Elisa.

Résultats. – La distribution des génotypes de polymorphismes 4G/5G et −844G/A obéit à l'équilibre de Hardy-Weinberg chez les sujets témoins. Une fréquence plus élevée du génotype 4G/4G, ainsi que des fréquences plus faibles de l'allèle −844A et des génotypes −844G/A et −844A/A ont été observées chez les patients avec RD, conférant ainsi respectivement une protection et une susceptibilité à la maladie. Les taux de PAI-1 étaient significativement élevés en présence du génotype 4G/4G comparés aux autres génotypes de PAI-1. Une différence significative des taux de PAI-1 entre les patients RD et SRD a été observée uniquement chez les porteurs de génotype −844G/G et chez les patients porteurs des haplotypes contenant 4G mais non pas chez ceux contenant 5G. Une distribution comparable des allèles, des génotypes, des haplotypes des 4G/5G

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et −844G/A, ainsi une similarité des taux plasmatiques du PAI-1 ont été observées aussi bien chez les patients avec rétinopathie diabétique proliférative (RDP) que chez ceux avec rétinopathie diabétique non-proliférative (RDNP), indiquant que ni les variants du PAI-1 ni les variations de son taux plasmatique ne sont associés à une sévérité de la RD. L'analyse multivariée, après ajustement sur de multiples covariables, a montré que les haplotypes 4G/−844A et 4G/−844G étaient respectivement associés négativement et positivement à la RD, mais non à la sévérité de RD (RDP versus RDNP).

Conclusion. – Cette étude identifie les modifications du taux de PAI-1 et la variation génétique au niveau du locus PAI-1 comme facteurs de risque de la RD, mais non de sa sévérité, ce qui pourrait servir de marqueur utile de l'augmentation de la susceptibilité à la RD.

Mots clés : Diabète ; Rétinopathie ; L'inhibiteur des activateurs du plasminogène-1 ; Polymorphisme ; PCR

1. Introduction

Type 2 diabetes (T2D) is a metabolic disease associated with serious micro- and macrovascular complications, including diabetic retinopathy (DR), a major cause of blindness among diabetic adults, that are aggravated by poor glycaemic control, hypertension and longer disease duration [1]. DR is associated with a strong genetic predisposition, highlighted by the familial clustering of DR [2,3] and the association of several gene polymorphisms with DR [4–11]. These include the aldose reductase [10,11], advanced glycation end-products receptor [6,7], adhesion molecules [8], and coagulation – and fibrinolytic – system gene polymorphisms, including the plasminogen activator system (PAS) [4,5,9].

PAS comprises distinct serine proteases (tissue-type plasminogen activator) and their inhibitors (plasminogen activator inhibitor [PAI]) [12], which control plasminogen activation [12,13]. High PAI-1 activity is associated with atherosclerosis and thromboembolism [14], and several polymorphisms within the PAI-1 gene influence PAI-1 levels [15]. These include the −675 4G/5G insertion-deletion mutation and the −844G/A single nucleotide polymorphism (SNP). While the 4G/5G and −844G/A variants are in strong linkage disequilibrium [16,17], they exert different effects on PAI-1 levels, with a strong association between 4G/5G – but not −844G/A – mutations and PAI-1 levels [16,17].

Table 1
Clinical characteristic of the study subjects.

Characteristic	DWR group ($n = 473$)	DR group ($n = 383$)	P
Gender (male/female)	219:254	169:214	0.535[a]
Age during study (years)	60.4 ± 9.4	60.9 ± 11.0	0.464[b]
Mean BMI (kg/m^2)	27.7 ± 5.2	28.0 ± 5.6	0.328[b]
Diabetes duration (years)	11.2 ± 5.1	11.9 ± 7.1	0.421[b]
Age of onset (years)	46.2 ± 9.9	46.0 ± 11.0	0.682[b]
Systolic BP (mmHg)	137.2 ± 29.5	143.9 ± 25.1	< 0.001[b]
Diastolic BP (mmHg)	81.1 ± 13.1	81.0 ± 12.2	0.928[b]
Glucose (mmol/L)	12.7 ± 5.1	12.5 ± 5.1	0.414[b]
HbA_{1c} (%)	9.7 ± 3.8	9.4 ± 3.3	0.261[b]
HDL (mmol/L)	1.05 ± 0.34	1.07 ± 0.40	0.644[b]
LDL (mmol/L)	3.81 ± 1.32	3.79 ± 1.45	0.889[b]
Total cholesterol (mmol/L)	5.12 ± 1.29	5.51 ± 1.56	< 0.001[b]
Triglycerides (mmol/L)	1.52 ± 1.12	2.09 ± 1.45	< 0.001[b]
PAI-1 antigen	30.78 ± 20.49	33.23 ± 20.18	0.094[b]

[a] Pearson's chi-square test.
[b] Student's *t*-test.

Elevated levels of PAI-1 are detected in the serum of diabetic individuals and experimental animals, and are implicated in retinal microvascular occlusion [18,19]. Insofar as the 4G/4G genotype is associated with increased PAI-1 gene transcription and elevated PAI-1 serum levels [20], it may be that the 4G/5G polymorphism is associated with DR pathogenesis. Previous studies of the association between the 4G allele and DR have yielded conflicting results, ranging from strong links [4,5] to no association [10,21,22], while others suggest that increased PAI-1 activity, independent of 4G/4G genotype, may be implicated in DR pathogenesis [18,22]. The aim of the present study was to investigate the role of the 4G/5G and −844G/A polymorphisms and changes in PAI-1 levels as risk factors of DR among adult T2D patients in Tunisia.

2. Subjects and methods

2.1. Subjects

This was a retrospective case-control study involving 856 unrelated adult T2D patients, recruited from the outpatients' endocrinology services at Farhat Hached University Hospital (Sousse) and Fattouma Bourguiba University Hospital (Monastir) in Tunisia. Written informed consent was obtained from all participants, and the study was carried out in accordance with the guidelines of the Helsinki declaration of 1975, and approved by the University of Monastir ethics committee. T2D diagnosis was based on clinical features; none of the patients had ever had ketoacidosis, and their initial T2D treatment included diet and/or oral antidiabetic drugs. Patients who required insulin had been treated with oral drugs for at least two years (Table 1).

For all study patients, demographic details were obtained and the patients' histories verified from clinic records where available. Venous blood samples were collected after an overnight fast for glucose, HbA_{1c} and serum lipid measurements. Total haemoglobin (Hb) and HbA_{1c} levels were measured by colorimetric and immunoturbidimetric methods, respectively, using a COBAS Integra 800 analyzer; the Hb-to-HbA_{1c} ratio yielded the percent HbA_{1c} levels. Blood pressure (BP) was measured twice using a mercury sphygmomanometer with participants in the sitting position following a 5-min rest; the mean of two readings was used. Hypertension was defined as BP readings greater than 140/90 mmHg and/or the use of antihypertensive medications.