

# -675 4G/5G and -844 G/A of Plasminogne Activator Inhibitor-1 (Pai-1) Gene Polymorphisms and Type 2 Diabetes Mellitus in Tunisia: Case-Control Study

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#### Abstract

Background: The plasminogen activator inhibitor-1 (PAI-1) is a puissant antifibrinolytic factor; plasma PAI-1 level is high in type 2 diabetes. 4G/5G polymorphism of PAI-1 gene is a major genetic determinant of plasma PAI-1 levels, with 4G carriers having high PAI-1 level than 5G, theses pose the question about relation T2 patients and those polymorphisms. The aim of this study was to determine the relationship between the polymorphisms -675 4G/5G and -844 G/A of PAI-1 gene and type 2 diabetes mellitus. Methods: A case control study of 491 diabetic and 400 healthy controls. Genotyping of the polymorphism -675 4G/5G was done by PCR-ASA (polymerase chain reaction, allele specific amplification), and the polymorphism -844 G/A was done with PCR-RFLP (restriction fragment length polymorphism), the allelic frequency is calculated with hardy-Weinberg law, the statistic analysis was done by SPSS version 10. **Results:** Higher frequencies of The genotypes 4G/4G (p = 0.01) and 4G/5G of polymorphism -675 4G/5G were seen in diabetic (p = 0.05) and higher frequencies of 5G/5G was seen in controls (p < 0.001). Higher frequencies of the genotype A/A (p < 0.01) and G/A (p = NS) of polymorphism -844 G/A was seen in diabetics and G/G was seen in controls (p = 0.01). Conclusion: Our study found association between 4G allele of -6754G/5G and A allele of -844 G/A of PAI-1 gene and having type 2 diabetes mellitus in Tunisian population.

# **Keywords**

Plasminogéne Activator Inhbitor-1 (PAI-1), -675 4G/5G Polymorphism,

-844 G/A Polymorphism, Type 2 Diabetes Mellitus

#### 1. Introduction

PAI-1 (plasminogen activator-inhibitor-1) is a powerful inhibitor of fibrinolysis. So it is a thrombogenic factor, it is incriminated in atherosclerosis [1] and vein thromboses or are high rate [2]. Type 2 diabetes is a disease with a very high cardiovascular risk [3] [4], where the level of PAI-1 Ag is increased [5]. This plasma level is determined by genetic polymorphisms [6] and environmental factors [7]. The elevation of plasma levels of PAI-1 during type 2 diabetes [7] [8] and the relationship between some polymorphisms of the PAI-1 gene and the plasma level of PAI-1 Ag raise the question of the possible association between these polymorphisms and type 2 diabetes (T2D).

## 2. Patients and Methods

This was a cross-sectional study, 491 type 2 diabetets patients and 400 healthy control was recruited after written consentement from endocrinology department at Farhat-Hachad hospital in Sousse-Tunisia, the study was approved by hospital ethic comity, inclusion criteria for patients was: known type 2 diabetes, exclusion criteria were: type 1 diabets, coagulation disorders, pregnancy, end stage chronic kidney disease, control are all clinically healthy personas who don't have chronic disease, all patients and contorls had clinical evaluation including (weight, high, BMI, Waist Circumference (WC)) and control had laboratory investigations include: Fasting blood glucose (FBG), Hb1Ac, cholesterol, triglyceride, HDL-cholesterol), urea, creatinemia, FGR was calculated with cockroft formula, genetic study was done or all patients and control; -675 4G/5G. PAI-1 gene promoter polymorphism genotyping was done by PCR-ASA (allele specific amplification) and -844 G/A polymorphism genotyping was done by PCR-RFLP (restriction fragment length polymorphism), allelic frequency was calculated with hardy-Weinberg law, statistical analyses was performed using SPSS version 10.0 software.

#### 3. Results

Our patients and controls are comparable in age and sex, with a sex ratio close to 1 in both cases, 74% of diabetic women and 76% of control women are postmenopausal. T2 diabetes patients have a significantly higher BMI. The control group has no metabolic abnormalities or impaired renal function. T2D have a significantly higher average total cholesterol, LDL-cholesterol, and triglycerides, and significantly lower mean HDL-cholesterol than controls, T2D had significantly higher urea and creatinine levels with clearance. Creatinine significantly lower, difference in serum uricemia is not significant (Table 1).

The Homozygous 4G/4G and heterozygous 4G/5G polymorphisms are significantly more common in T2D patients than in controls (16.7% and 49.1% versus

	Patients n = 491	Controls n = 400	Р
Age (Mean ± SD)	$58.32 \pm 10.5$	$58.2 \pm 8.5$	NS
Sex-ratio	0.92	1.05	NS
Menopause n (%)	191 (74.1)	180 (76)	NS
BMI	$27.5 \pm 5.4$	$23.4\pm2.2$	< 0.001
FBG (mmol/l)	$12.49\pm5.37$	$5.1 \pm 0.6$	< 0.001
Triglyceride (mmol/l)	$1.89 \pm 1.55$	$1.16\pm0.56$	< 0.001
Cholesterol (mmol/l)	5.79 ± 1.5	$4.6 \pm 1.23$	< 0.001
HDL-CHT (mmol/l)	$0.96\pm0.24$	$1.2 \pm 0.32$	<0.001
LDL-CHT (mmol/l)	$4.4\pm1.1$	$2.5 \pm 1.66$	< 0.001
Creatine (µmol/l)	$101.85\pm82$	82.8 ± 26.8	<0.001

Table 1. Clinicals and biologicals of T2D patients and controls.

FBG = fast blood glucose.

9.5% and 39.8%, respectively). Homozygous genotype 5G/5G is significantly more common in controls (50.8% versus 34.2%), the 4G allele is significantly more common in T2D while the 5G allele is more common in controls (Table 2).

The homozygous A/A genotype is significantly more common in T2D than in controls (18.7% versus 9.3%). The heterozygous genotype G/A is also more common in T2D (47.9% versus 44%). 8%), but the difference is not significant. The homozygous genotype G/G is significantly more common in controls (46% versus 33.4% in T2D). Allele A is more common in T2D and the G allele is more frequent in controls (p < 0.001) (Table 3).

## 4. Discussion

PAI1 is the most powerful physiological inhibitor of fibrinolysis. PAI-1 is part of "serpins" and is also called serpin 1 [9].

The PAI-1 gene is located at chromosome 7, in position q 21.3-q 22 [6].

Approximately 100 polymorphisms of the PAI-1 gene are currently identified [10].

The most studied polymorphism is a deletion insertion of a 4G or 5G guanosine localized at 675 bp (base pairs) upstream of the transcription start site (-675) [11] [12].

A second polymorphism at the promoter region of the PAI-1 gene is the G/A substitution at position -844 [12] [13].

The elevation of plasma PAI-1 Ag in type 2 diabetes is confirmed by most studies. Several studies have shown that the 4G allele of the -675 4G/5G polymorphism is associated with a high level of PA-1 have also shown that this allele is associated with an android fat distribution, suggesting the possible involvement of this allele in susceptibility to type 2 diabetes.

Hoffestedt *et al.* [14] found that the RR of being obese in subjects with the 4G allele was 1.9.

Génotyps n (%)	T2D	Controls	р
4G/4G	82 (16.7%)	38 (9.5%)	0.001
4G/5G	241 (49.1%)	159 (39.8%)	0.005
5G/5G	168 (34.2%)	203 (50.8%)	< 0.001
4G/5G alleles %	41.1/58.9	29.4/70.6	< 0.001

**Table 2.** And alleles distribution of –675 4G/5G polymorphism in T2D patients and controls.

**Table 3.** And alleles distribution of -844 G/A polymorphism in T2D patients and controls.

Génotyps n (%)	T2D	Controls	р	
A/A	92 (18.7%)	37 (9.3%)	< 0.001	
G/A	235 (47.9%)	179 (44.8%)	NS	
G/G	164 (33.4%)	184 (46%)	0.001	
G/A alleles%	42.6/57.4	31.6/68.4	<0.001	

The relationship between the 4G allele and obesity was observed in both sexes but was more pronounced in humans.

Lopes *et al.* [15] also found an association between 4G/5G polymorphism and obesity.

Mc Cormack [16] and Sartori *et al.* [17], Sola [18] found no relation between 4G/5G polymorphism and obesity.

The authors of the Quebec Family Study [19] found an association between 4G/4G genotype and BMI, fat mass, hip circumference (TH), total abdominal fat and subcutaneous abdominal fat, only in women.

Naran [20] found an association between 4G/4G genotype and high TT in white subjects.

Paradoxically, Van Hermelen *et al.* [21] found a higher BMI in subjects with genotype 5G/5G compared to genotype 4G/4G. However, they did not find any relationship between 4G/5G polymorphism and TT/TH ratio, insulinemia, triglyceride levels, fat cell volume, PAI-1 adipose secretion, or plasma activity of PAI-1.

The association of the -675 4G/5G polymorphism with type 2 diabetes has been the subject of several studies.

A recent metanalysis [22] of 14 studies (12 Asian studies, 2 studies of Caucasians) found a significant association between the 4G allele and type 2 diabetes, which is not significant for Caucasians.

A recent Malaysian study [23] found a significant association between the 4G allele and the prevalence of diabetes.

The Framingham Offspring Study [24] is a prospective study that included 2169 Caucasians., The authors did not find any relationship between this polymorphism and the incidence of type 2 diabetes or insulinoresistance markers.

Zietz [25], found a distribution of 4G and 5G alleles in diabetics close to that

of the general population. Lopes *et al.* [15] found no significant difference in the distribution of genotypes and alleles of the -675 4G/5G polymorphism between diabetics and non-diabetics.

Naran et al. [20] have similar results.

Broach [26] found a genotype frequency of 4G/4G of 20.3%, genotype 5G/5G of 26.6% and genotype heterozygote 4G/5G of 53.1%, in 177 Caucasian Caucasian type 2 diabetics. Nagi [27] *et al.* comparable frequencies in diabetic Pimas Indians.

McCormack [16] and Mansfield [28] in their case-control studies found no differences in the frequencies of the different genotypes and alleles of the -675 4G/5G polymorphism between diabetics and non-diabetics.

An Austrian study found a significant relationship between the 4G allele and the discovery of gestational diabetes genotype 4G/4G and maternal age emerged as two independent risk factors for gestational diabetes [29].

In our study, we found a significant difference in the distribution of different genotypes and alleles between diabetics and controls. The frequency of the 4G allele was significantly higher in diabetics compared to controls (41.2% versus 29.4%). It was the same for the homozygous genotype 4G/4G (16.7% versus 9.5%).

The frequency of the 5G allele was higher in controls compared to diabetics. (70.7% versus 58.7%). It was the same for the homozygous genotype 5G/5G (50.8% versus 34.2%).

Our results support an association between the 4G allele and type 2 diabetes. These results are in agreement with literature data in non-Caucasian populations and may be explained by the frequency of obesity and abnormalities. our group of diabetics. While our controls are free from obesity and metabolic abnormalities.

A Tunisian study [30] found a relationship between the 4G allele and diabetic retinopathy, which is consistent with findings from other studies [27] [31]. 2 other Tunisian studies found a relationship between this allele 4G and stroke [32] and MI [33].

The -844 G/A polymorphism has been less studied in type 2 diabetics.

A Tunisian study found a relationship between the A allele and the IDM [33].

In the Lopes study [15], there was no significant difference in the distribution of the different genotypes and alleles of the -844 G/A polymorphism between diabetics and non-diabetics, but they found that obese subjects carrying the genotype A/A had higher blood glucose and insulin levels than the G allele carriers.

A Mexican study of 100 children found an association between the A allele and the metabolic syndrome [34].

In our series, the prevalence of the homozygous A/A genotype was higher in diabetics compared to controls (18.7% versus 9.3%). It was the same with that of the allele A (42.6% versus 31.7%).

In controls, the homozygous genotype G/G was more frequent (46% versus 33.4% in diabetics) as well as the G allele (68.3% versus 57.4%).

The strong points of our study: this is the first African study outside of South Africa that studied the relationship between these 2 polymorphisms and type 2 diabetes and the large size of our sample and the fact that it studied the polymorphism -844 G/A rarely studied. But the study is limited by the transversal character as most studies, the realization of longitudinal studies with a large workforce is necessary to verify the role of these polymorphisms in the occurrence of diabetes type 2.

## **5.** Conclusion

Our work shows an association between the 4G polymorphism allele -675 4G/5G and the A8 -844 G/A polymorphism allele of the PAI-1 gene and type 2 diabetes in the Tunisian population.

#### **Conflicts of Interest**

All authors declare no conflicts of interest.

## **Author's Participation**

All authors had participated actively in manuscript realization.

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