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The Plasminogen Activator Inhibitor 1 4G/5G Polymorphism and the Risk of Alzheimer's Disease

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Abstract

Objective: The aim of this study was to determine whether plasminogen activator inhibitor 1 (PAI-1) is associated with the risk of Alzheimer's disease (AD) in Tunisian patients. **Design and Methods:** We analyzed the genotype and allele frequency distribution of the PAI-1 polymorphism in 60 Tunisian patients with AD and 120 healthy controls. **Results:** The results show a significantly increased risk of AD in carriers of the 4G/4G and 4G/5G genotypes versus the wild-type 5G/5G genotype (4G/4G: 28.33% in patients vs 10.0% in controls; $P < 10^{-3}$; OR = 8.78; 4G/5G: 55.0% in patients vs 38.33% in controls; OR = 4.45; $P < 10^{-3}$). The 4G allele was also more frequently found in patients compared with controls; $P < 10^{-3}$; OR = 3.07. For all participants and by gender, homozygotic carriers (4G/4G) were at an increased risk of AD over heterozygotes and women were at an increased risk over their male genotype counterparts. The odds ratio for AD among 4G/4G carriers for any group was approximately twice that of heterozygotes in the same group. Women homozygotes ranked highest for AD risk (OR = 20.8) and, in fact, women heterozygotes (OR = 9.03) ranked higher for risk than male homozygotes (OR = 6.12). **Conclusion:** These preliminary exploratory results should be confirmed in a larger study.

Keywords

plasminogen activator inhibitor 1, risk factor, genotyping, Alzheimer's disease

Introduction

Senile plaques composed mainly of amyloid-β (Aβ) are particularly important in the pathology of Alzheimer's disease (AD).[1] These deposits trigger prolonged inflammation, neuronal death, and progressive cognitive decline.[2]

Plasmin is one of several proteases that may regulate Aβ levels in the brain. Plasmin formation is governed by plasminogen activators (PAs) that cleave the pro-enzyme plasminogen to the active protease, plasmin. There are 2 PAs: tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). It is clear that tPA has multiple roles in both physiological and pathological situations in the brain, whereas uPA has generally been described as being absent from the normal central nervous system (CNS). However, uPA is known to be stress responsive and is induced during inflammation. Urokinase plasminogen activator is expressed in the inflamed CNS in a model of experimental allergic encephalopathy and in cerebral ischemia in rodents and humans.[3] Tissue plasminogen activator has been shown to be highly expressed in areas of the brain where plaques are deposited, but tPA induction in these cases does not lead to efficient plasmin generation and Aβ clearance.[4] Several mouse models agree that increasing Aβ levels occur concurrently with the overproduction of plasminogen activator inhibitor 1 (PAI-1) and decreasing tPA activity.[2,5-7] Liu et al found that PAI-1 expression/activity contributes importantly to Aβ accumulation during aging and in AD probably by inhibiting plasminogen activation and thus inhibiting Aβ degradation.[7] Oh et al found that tPA-mediated plasmin activity declines throughout the brain causing Aβ deposition during aging, and the Aβ deposits locally attract the cluster of tPA and/or PAI-1 around their deposits to competitively determine tPA/plasmin-mediated Aβ proteolysis.[6]

Proteases in the PA system are inhibited by members of the serpin (serine protease inhibitors) family, one of which is PAI-1. Plasminogen activator inhibitor, found in a variety of tissues,[8] irreversibly inhibits both tPA and uPA and their activity and

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decreases the level of plasmin.[9] The inhibition of plasminogen activity may then be implicated in the pathogenesis of AD. Neuroserpin is a major inhibitor of tPA in the human brain.[10-12]

The gene coding for the PAI-1 has several polymorphic loci, among which the most studied is the 4G/5G insertion/deletion polymorphism containing either 4 or 5 (4G/5G) guanine bases at −675 within the promoter region of the human *PAI-1* (*SERPINE1*) gene.[13] The 4G/5G polymorphism is located upstream from the start of the transcription site of the PAI-1 gene and can cause an increase in PAI-1 gene transcription and expression.[14] One guanine nucleotide deletion in the PAI-1 promoter results in the removal of 1 binding site for a DNA-binding protein that behaves as a transcriptional repressor; thus, the 4G allele will have a higher transcriptional activity than the wild-type 5G allele. It has been observed that the promoter polymorphisms in the PAI-1 gene can impact PAI-1 expression and have been implicated as a risk factor for several vascular diseases to some extent.[15-17] However, their precise role remains controversial. Moreover, there are only a few studies on the association between the PAI-1 polymorphism and AD susceptibility and those results are inconsistent.[18,19] There are additional studies that demonstrate this inconsistency of brain PAI-1 levels in patients with AD. However, the difference between their results and some other studies may be related to the patient's age. Fabbro and Seeds[11] saw no difference in PAI-1 levels in AD brain tissues of those patients with a mean age of 64, while Magistri et al saw a significant difference in brain PAI-1 RNA expression in an AD population with a mean age of 82 as compared to controls. This suggests that the PAI-1 increase is more visible in older patients with AD.[20]

Therefore, the present study has been undertaken to better understand the relationship between the insertion/deletion (4G/5G) polymorphism in the promoter region of the PAI-1 gene and AD in Tunisian patients.

Patients and Methods

Study Population

We studied 60 patients with AD (20 females and 40 males) recruited from the Department of Neurology at the Military Hospital of Tunis. The diagnoses of probable AD were based upon the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association clinical diagnostic criteria and the *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition).[21,22] All participants underwent a complete clinical investigation that included medical history, neurological and neuropsychological examinations (Mini-Mental State Examination [MMSE],[23] clock-drawing tests, the 5-word test, auditory verbal learning test, and Frontal Assessment Battery), screening laboratory tests, and neuroimaging consisting of computed tomography (CT) scan and/or magnetic resonance imaging (MRI). MRI scans displayed substantial reduction in the medial temporal lobe and hippocampal volume of patients with AD as compared to controls.

Additionally, the control group consisted of 120 age- and gender-matched participants (46 females and 74 males) with diverse Tunisian origin similar to that of the patients and chosen based on their medical history and physical examination. Their cognitive function was assessed using the MMSE examination, and they did not exhibit signs of dementia. No family history of AD or dementia was reported in controls. All participants in this study or their guardians had given their fully informed consent. The study protocol was approved by the ethics committee of the Military Hospital of Tunisia and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Laboratory Methods

Genomic DNA was extracted from peripheral blood leukocytes with DNA extraction kit (QIAamp blood kit; Qiagen GmbH, Hilden, Germany), according to the manufacturer's protocol. Genotyping of the −675 4G/5G polymorphism in the PAI-1 promoter region was carried out by polymerase chain reaction using oligonucleotide sense primer 5′-CACAGAGA-GAGTCTGGCCACGT-3′ and the antisense primer 5′CCAACAGAGGACTCTTGGTCT-3′. The amplified DNA was incubated with the specific restriction enzyme BslI, and the cleaved fragments were analyzed with electrophoresis on a 2% agarose gel stained with ethidium bromide.

Statistical Analyses

All analyses related to the case–control study were performed using the Statistical Package for the Social Sciences v.16 (IBM, Armonk, New York). Data on quantitative characteristics are expressed as means (SD). Differences between cases and controls were evaluated by using the χ^2 test or Fisher exact test for qualitative variables. In addition, the odds ratio (OR) and 95% confidence intervals were calculated. Probability (P) values <.05 were considered statistically significant. The power of statistical results were calculated using G*Power 3.1.7 (University of Düsseldorf).[24]

Results

The demographic data of patients are presented in Table 1. The mean age (SD) of the cases and controls was 75.18 (5.31) years and 72.94 (5.0) years, respectively. There were no significant differences in any of the mean values of blood chemistry including triglycerides and cholesterol between the AD and control groups. The MMSE scores differed significantly between patients with AD and controls ($P < .001$).

The 4G/5G PAI genotype frequencies were found to be in Hardy-Weinberg equilibrium in both the patient ($\chi^2 = 0.73$) and control populations ($\chi^2 = 0.8$). The carriers of the 4G allele, 4G/4G and 4G/5G considered separately, were compared against those carriers of the wild-type genotype (5G/5G). The analysis of the participants together and by gender revealed 2 shared characteristics: the 4G allele was a risk factor

Table 1. Comparison of Clinical Variables in Patients With Alzheimer's Disease and Controls.[a]

Clinical Variable	Patients (n = 60), n (%)	Controls (n = 120), n (%)	*P* Value
Gender: female/male	20 (33.3)/40(66.6)	46 (38.3)/74(61.6)	–
Age, years (SD)	75.18 (5.31)	72.94 (5.0)	–
Age of onset, years (SD)	69 (4.48)	–	–
MMSE (range)	14 (6-22)	28 (>26)	**$<10^{-3}$**
Tobacco use	12 (20)	30 (25)	.45
Diabetes	13 (21.6)	18 (15)	.26
Hypertension	15 (25)	20 (16.6)	.18

Abbreviations: MMSE, Mini-Mental State Examination; SD, standard deviation.
[a]Significant *P* values in bold.

Table 2. Distribution of PAI-1 4G/5G Genotypes and Alleles in Patients With AD and Controls (All Participants and by Gender).[a]

	All Participants					
Genotype PAI-1 (rs1799889)	Patients (n = 60), n (%)	Controls (n = 120), n (%)	χ^2	OR (95% CI)	*P* Value	Power (%)
5G/5G	10 (16.66)	62 (51.66)	–	–	–	–
4G/5G	33 (55.00)	46 (38.33)	14.38	**4.45 (1.99-9.93)**	**$<10^{-3}$**	**97**
4G/4G	17 (28.33)	12 (10.00)	21.12	**8.78 (3.24-23.8)**	**$<10^{-3}$**	**99**
5G	53 (44.16)	170 (70.83)	24.13	**3.07 (1.95-4.84)**	**$<10^{-3}$**	**99**
4G	67 (55.83)	70 (29.16)				
	Females					
	n = 20, n (%)					n = 46, n (%)
5G/5G	2 (10.00)	25 (54.35)	–	–	–	
4G/5G	13 (65.00)	18 (39.13)	8.97	**9.03 (1.81-45.0)**	**.006**	**86**
4G/4G	5 (25.00)	3 (6.52)	*f*	**20.8 (2.73-159)**	**.003**	**89**
5G	17 (42.50)	68 (73.91)	12.00	**3.83 (1.76-8.37)**	**$<10^{-3}$**	**92**
4G	23 (57.50)	24 (26.09)				
	Males					
	n = 40, n (%)					n = 74, n (%)
5G/5G	8 (20.00)	37 (50.00)	–	–	–	–
4G/5G	20 (50.00)	28 (37.84)	6.3	3.30 (1.27-8.59)	**.01**	70
4G/4G	12 (30.00)	9 (12.16)	10.57	**6.12 (1.95-19.5)**	**.001**	**88**
5G	36 (45.00)	102 (68.92)	12.44	**2.71 (1.55-4.75)**	**$<10^{-3}$**	**94**
4G	44 (55.00)	46 (31.08)				

Abbreviations: CI, confidence interval; OR, odds ratio.
[a]4G/*G genotypes versus 5G/5G. Significant *P* values are in bold. *f*, Statistics calculated with Fisher exact test. OR values detected with a minimum of 80% power are in bold. (Power calculated using G*Power 3.1.7.).

for AD and homozygous carriers were at a risk of AD measured at approximately double the odds as heterozygous carriers (all participants: 4G/5G vs 5G/5G: $P < 10^{-3}$, OR = 4.45; 4G/4G vs 5G/5G: $P < 10^{-3}$, OR = 8.78). The data by gender also revealed a significant bias toward a higher risk for female heterozygotes/homozygotes over their male counterparts (4G/5G vs 5G/5G: women OR = 9.03; men: OR = 3.30; 4G/4G vs 5G/5G: women OR = 20.8; men: OR = 6.12). Female carriers of the genotype 4G/*G have a risk of AD that is approximately a 3-fold increase in the odds as compared to men with the same genotype (Table 2).

Discussion

In the present study, we analyzed the polymorphism in the promoter regions of the PAI-1 gene at position −675 base pair (bp; 4G/5G) upstream from the start of transcription. An association between this polymorphisms and AD was evident in our population. To our knowledge, this is the first study to associate the PAI-1 4G/5G polymorphism with AD in the Tunisian population.

The gene coding for PAI-1 has several polymorphic loci, among which the most studied is the 4G/5G insertion/deletion polymorphism containing either 4 or 5 (4G/5G) guanine bases at −675 within the promoter region of the human *PAI-1* (*SERPINE1*) gene.[14] Both alleles of this SNP can bind a transcriptional activator, whereas the 5G allele binds a repressor protein at an overlapping site. Therefore, homozygosity for the 4G allele renders this negative regulator unable to act, resulting in greater transcription of the *PAI-1* gene, while heterozygotes show intermediate phenotype.[25] This may explain the increase in the risk of AD in our population of homozygote carriers of the 4G allele over heterozygotes. In humans, increased PAI-1

expression has been observed near amyloid deposits or sites with inflammatory responses in the brains of patients with AD.[26] Hino et al, concurring with research by Rebeck et al, describe deposition of PAs and PAI-1 in senile plaques.[27,28] Thus, the gene coding PAI-1 is a functional candidate for AD-related susceptibility genes.

It was known that brain plasmin enhanced Aβ degradation, while the plasmin and its activity were decreased in AD brains.[29,30] Since Aβ is a component of plaques that are often associated with AD, the deposited material has been hypothesized to be a pathogenic factor.[31]

Plasminogen activator inhibitor 1 inhibits the activity of tPA and uPA and thus inhibits the conversion of plasmin from inactive plasminogen. Overexpression of PAI-1 inhibits the activity of the tPA/plasminogen system and results in a reduced ability to degrade Aβ. In addition, the increased Aβ accumulation could lead to further PAI-1 induction as there is evidence suggesting that expression of PAI-1 was observed near amyloid deposits or at the sites with inflammatory response in the brain of patients with AD.[26] Amyloid beta peptide, a major hallmark of AD, has also been found to be cleaved by the tPA/plasminogen proteolytic cascade in vitro and in vivo.[32,33]

There is scant research on the association between the PAI-1 polymorphisms and AD susceptibility and the data have been disparate. In a study of Korean participants, the authors observed an increase in the blood levels of PAI-1 in patients with AD and demonstrate that PAI-1 in the plasma is a useful early-stage AD biomarker.[34] However, other studies failed to support a relationship between the PAI-1 gene promoter and AD.[18,35]

Shibata et al had reported that a single guanine deletion/insertion (4G/5G) polymorphism at −675 bp upstream from the start of transcription in SERPINE1 was associated with AD in patients from Northern Europe. However, this association was not confirmed in follow-up studies using an independent Canadian case–control cohort and 2 familial AD data sets of Northern European and Caribbean Hispanic origin.[19]

The main limitation of the present study was the small sample size. Larger studies will be required to confirm the results and to better understand the consequences of the PAI-1 4G/5G polymorphism on AD as they relate to other ethnic groups. Furthermore, the small sample size restricted the ability to assess potentially varying PAI-1 effects according to AD subtype. Larger studies where patients can be grouped by AD subtype are required to address this point. Additionally, a study of the correlation of PAI-1 blood levels with genotypes would be of interest.

In conclusion, the present study shows for the first time that the 4G/5G insertion/deletion polymorphism in the promoter region of the SERPINE1 gene is associated with AD in Tunisian patients. Further work is required to discern among possible differing findings in diverse populations.

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Declaration of Conflicting Interests

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