PAI and TPA gene polymorphisms in multiple sclerosis

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PAI and TPA gene polymorphisms in multiple sclerosis

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Multiple sclerosis (MS) is an immune-mediated chronic inflammatory demyelinating disease of the central nervous system. It manifests as acute focal inflammatory demyelination and axonal loss with limited remyelination and results in the chronic multifocal sclerotic plaques. Previously published data showed impaired fibrinolysis in MS. Tissue plasminogen activator t-PA is a serine protease that catalyses the activation of plasmin, which mediates the effects of fibrinolytic system. Alu insertion/deletion (I/D) genetic polymorphism in TPA gene in MS patients has not been analysed previously. The major inhibitor of t-PA is plasminogen activator inhibitor-1 (PAI-1). Its gene expression is modulated by functional genetic polymorphism in the promoter (4G/5G). In the present study, an association of two genetic polymorphisms with MS, its progression and subtype were analysed. TPA DD/PAI-1 4G4G genotype combination has reached a borderline significance for reduced risk for MS (OR = 0.543, 95% CI 0.301–0.978, $P = 0.04$), suggesting a gene–gene interaction. The explanation for this interaction may be a complex interplay between these two pleiotropic proteins within the brain tissue and in plasma. *Multiple Sclerosis* 2008; **14**: 243–247. http://msj.sagepub.com

Key words: gene polymorphism; multiple sclerosis; PAI 4G/5G; TPA Alu I/D; susceptibility gene

Introduction

Multiple sclerosis (MS) is an immune-mediated chronic inflammatory demyelinating disease of the central nervous system. It manifests as acute focal inflammatory demyelination and axonal loss with limited remyelination and results in the chronic multifocal sclerotic plaques [1]. The initiating event in MS is unknown, but relevance of the gentic contribution is widely accepted. The underlying cause of the disease is believed to be independent or epistatic interaction of combination of different polymorphisms, each of which has a small contribution to final effect on onset and progression of the disease [1,2].

Previously published data showed impaired fibrinolysis in MS. The co-localization of tissue-type plasminogen activator (t-PA) to demyelinated axons as well as fibrinogen deposits was shown in chronic inflammation characteristic of MS suggesting an association with axonal damage [3]. Fibrinolysis is regulated with the balance between plasminogen activators and inhibitors. The major inhibitor of t-PA is plasminogen activator inhibitor-1 (PAI-1). Due to formation of complexes of t-PA with inhibitors like PAI, the fibrinolytic potential in demyelinating MS lesions is greatly diminished [4]. Moreover, it has been hypothesized that limited availability of t-PA in MS lesions because of the formation of t-PA/PAI-1 complexes reduces the capability of t-PA receptors to generate plasmin, which further diminishes fibrinolytic capacity in active MS lesions and possibly leads to axonal damage [5].

On the other hand, it may represent a mechanism to remove fibrin deposits, since PAI-1 and t-PA form stable 1:1 complexes with high affinity, which are then cleared through internalization by macrophages. Also, it has been suggested that plasmin-mediated clearance of fibrin in experimental autoimmune encephalomyelitis (EAE), an animal

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model of MS, may limit the extent of immune infiltration and neuroinflamation [6].

t-PA is a serine protease that catalyses the conversion of the zymogen plasminogen to the active enzyme plasmin, which mediates the effects of fibrinolytic system. Alu insertion/deletion (I/D) genetic polymorphism in TPA gene in MS patients has not been analysed previously.

Gene expression of major t-PA inhibitor, PAI-1, is modulated by common functional genetic polymorphism in the PAI-1 promoter (4G/5G). Individuals with the 4G/4G genotype have increased plasma PAI-1 concentrations [7]. Genotype 5G5G, which is associated with a lower production of PAI-1, and frequency of alel 5G were previously published to be significantly increased in woman with MS [8].

The objective of the present study was to assess the potential association of two genetic polymorphisms (I/D polymorphism of the TPA gene, 4G/5G polymorphism of PAI-1 gene) with increased risk for MS, progression and subtype of the disease.

Materials and methods

Patients and control subjects

After approval by the local ethics commitee and written informed consent, blood was collected from 333 MS patients (Table 1) recruited by collaborating genetic centers from Slovenia (157 patients) and Croatia (176 patients). All patients had clinically definite MS according to the Poser criteria [9]. Disability was assessed using Kurtzke's Expanded Disability Status Scale (EDSS) [10]. The rate of accumulation of neurological disability was expressed by the progression index (PI) of disease and was calculated as ratio EDSS/disease duration (years).

Three hundred and sixty eight unrelated ethnically matched healthy blood donors with no history of MS in their families represented the control group.

PAI and TPA genotyping

Genomic DNA was extracted from peripheral blood leukocytes according to standard protocols. PAI-1 4G/5G and TPA Alu I/D polymorphisms were evaluated as described previously [11,12].

Statistical analysis

Allele frequencies were calculated by gene counting, and deviations from those estimated by the Hardy–Wienberg equilibrium were tested using the $\chi 2$ test. The significance of the difference of observed alleles and genotypes between MS patients and control subjects was determined using the $\chi 2$ test. Relationship between the genotypes of PAI-1 and TPA polymorphisms and the disease progression and age at the onset of the disease of MS patients was tested by one-way ANOVA test and logistic regression analysis.

Due to previously published results that insertion allele of TPA confined increased risk of myocardial infarction and possibly impaired fibrinolytic capacity, odds ratios (OR) for this polymorphism were computed assuming a dominant model for the I allele [13]. In line with this, carriers of the rare PAI-1 5G allele were compared with subjects homozygous for the 4G allele. $\chi 2$ test and Fisher exact probability test were performed to estimate OR and 95% confidence intervals (CI). The results were considered statistically significant at $P < 0.05$ using a two-tailed test.

Our analyses concerned the whole study group and were, subsequently, also stratified by the two main categories of the disease at onset – relapsing remitting (RRMS) and primary progressive (PPMS) multiple sclerosis. In each stratum, cases were compared with the entire control group.

Results

The observed distribution of genotypes showed no significant difference when compared with those

Table 1 Clinical characteristics of MS patients ($N = 333$)

	All (333)	Women (239)	Men (94)
Age[a]	42.85 ± 12.52(16–78)	42.50 ± 12.35(20–74)	43.71 ± 12.98(16–78)
Age at onset[a]	29.41 ± 8.91 (10–54)	29.44 ± 9.03 (12–53)	29.34 ± 8.64 (10–54)
Initial course			
RRMS	308 (92.5%)	223 (93.3%)	85 (91.4%)
PPMS	25 (7.5%)	16 (6.7%)	9 (9.6%)
Duration (years)[a]	13.75 ± 10.77 (1–52)	13.44 ± 10.39 (1–52)	14.51 ± 11.60 (1–47)
EDSS[a,b]	4.26 ± 2.48 (0.5–10)	4.10 ± 2.37 (0.5–10)	4.68 ± 2.70 (1–10)
PI[a,c]	0.38 ± 0.30 (0.06–2.17)	0.38 ± 0.29 (0.06–2.17)	0.40 ± 0.33 (0.08–2.17)

[a]Mean ± SD.
[b]Disability was assessed by using Kurtzke's Expanded Disability Status Scale (EDSS).
[c]PI, progression index, is expressed as the ratio EDSS/disease duration (years).

Table 2 Frequency of PAI 4G/5G and TPA Alu I/D genotypes and alleles in MS patients and controls

Genotype/allele	MS patients *N* (%)	Controls *N* (%)	OR (95% CI)[a]	*P*-value
PAI 4G/5G				
4G4G	86 (25.8)	109 (29.6)	0.83 (0.59–1.21)	0.263
4G5G	178 (53.5)	200 (54.4)	0.96 (0.72–1.30)	0.812
5G5G	69 (20.7)	59 (16.0)	1.37 (0.93–2.01)	0.109
Total	333	368		
Allele 4G	350 (52.6)	418 (56.8)	0.84 (0.68–1.04)	0.111
Allele 5G	316 (47.4)	318 (43.2)	1.19 (0.96–1.46)	0.111
TPA I/D				
DD	74 (22.2)	101 (27.5)	0.75 (0.53–1.07)	0.111
ID	153 (45.9)	146 (39.7)	1.29 (0.96–1.74)	0.094
II	106 (31.8)	121 (32.8)	0.95 (0.69–1.31)	0.767
Total	333	368		
Allele D	301 (45.2)	348 (47.3)	0.92 (0.75–1.13)	0.433
Allele I	365 (54.8)	388 (52.7)	1.09 (0.88–1.34)	0.433

[a]OR (95% CI).

predicted from the Hardy–Weinberg equilibrium for either patients or controls (P > 0.05).

There was no significant difference between MS patients and control subjects in the distribution of TPA I/D and PAI-1 4G/5G genotypes or in the allelic frequencies (Table 2). Different polymorphisms were equally distributed among RRMS and PPMS (data not shown). Also, when data were stratified in relation to gender, there was no statistically significant difference in the distribution of genotypes and alleles of both tested genes.

TPA DD/PAI-1 4G4G genotype combination has displayed a borderline significance for reduced risk for MS (OR = 0.543, 95% CI 0.301–0.978, $P = 0.04$) (Figure 1).

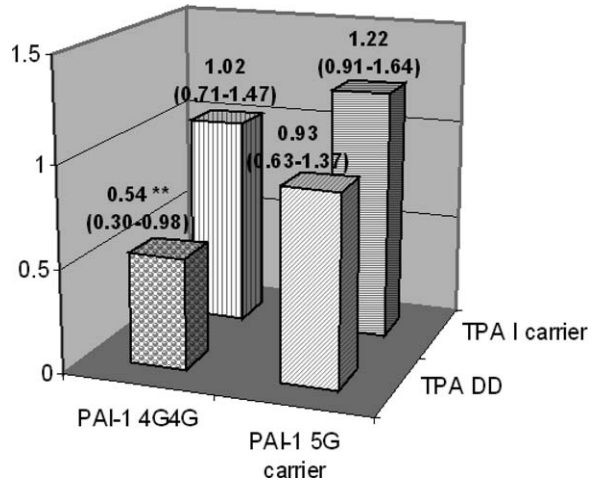

Figure 1 ORs and 95% CIs for combination of genetic variants. Number of cases/controls for the different combinations were: 18/35 for PAI-1 4G4G/tPA DD; 56/66 for PAI-1 5G carrier/tPA DD; 68/74 for PAI-1 4G4G/tPA I carrier; 191/193 for PAI-1 5G carrier/tPA II; $^{**}P < 0.05$.

The statistical power of our study is more than 80% ($\alpha = .05$, one-tailed), assuming at least 1.6 difference in genotype frequency.

Discussion

TPA DD/PAI-1 4G4G genotype combination might confer a reduced risk for MS. Alone, neither TPA I/D nor PAI-1 4G/5G gene polymorphisms showed significant association with MS.

To our knowledge, this is the first study analysing Alu I/D polymorphism in MS. Previously reported data showed defective fibrinolysis in MS with PAI-1 being one of the major enzymes included. The effects of fibrinolytic system are mediated by plasmin, the protease generated by the action of plasminogen activators on the inactive precursor plasminogen. The major inhibitor of t-PA is PAI-1. The genetic polymorphism 4G/5G modulates the expression of PAI-1 gene and individuals with the 4G/4G genotype have increased plasma PAI-1 concentrations. The results of our study did not show any significant association of PAI-1 4G/5G genotypes and MS or age of onset of progression of the disease.

The contribution of PAI-1 promoter polymorphisms to MS was also tested previously [8]. The 5G5G genotype was associated with increased risk of MS in women. We did not confirm this observation, neither for the whole MS group, nor when the group was stratified according to gender. However, there was a trend toward increased risk of MS in group with genotype 5G5G (OR = 1.39), but not statistically significant. The OR value was somewhat higher in women (OR = 1.48), but still it has not reached the significance ($P = 0.08$). It is believed that multiple genetic and environmental factors

influence an individual's risk for MS. For genetically complex diseases, risk alleles do not determine the presence or onset of the disease, they merely confer some level of probability or higher risk for developing certain disease [14]. Furthermore, the presence of high-risk allele may only mildly be associated with the disease and weakly penetrant alleles are present at high frequency in the general population. Therefore, large groups of patients are needed to obtain more objective data on high-risk alleles.

Also, our study is the first report describing the TPA Alu I/D genetic polymorphism in MS patients. It has been shown that PAI-1 and t-PA form stable 1:1 complexes with high affinity, and it has been suggested that due to these complexes, there is limited availability of t-PA in MS lesions [4,5]. This could lead to reduced capability of t-PA receptors to generate plasmin, which further diminishes fibrinolytic capacity in active MS lesions and possibly leads to axonal damage. The Alu polymorphism was previously shown not to be directly associated with t-PA plasma levels. Also, the basal endothelial t-PA synthesis was reported not to be influenced by the TPA Alu polymorphism [15]. However, the systemic concentration of t-PA is dependent not only on both secretion and clearance but also on its rate and degree of complex-formation with PAI-1 [16]. This is due to the fact that t-PA/PAI-1 complex is cleared at a slower rate than free t-PA [17]. Therefore, an increased plasma concentration of PAI-1 will be paralleled by an increased plasma concentration of total t-PA and, consequently, the systemic plasma level of t-PA is unlikely to directly reflect its secretion rate [12]. Also, these findings do not exclude the existence of an association between this polymorphism and the endogenous fibrinolytic capacity. Namely, the circulating levels of t-PA might not reflect the fibrinolytic capacity at the moment and site of thrombus formation. In addition, the Alu repeat insertion may be closely linked to a mutation at or near the TPA gene that produces a functional effect [13]. So far, it has not been elucidated whether or not genetic variation of PLAT genetic locus, which codes for t-PA, is a potential predictor for plasma t-PA levels. Indeed, Ladenvall *et al*. reported significant associations between some very rare single nucleotide polymorphisms (SNPs) and plasma t-PA, but they also indicated that results should be interpreted with caution since associations were tested in different models [18]. Moreover, these rare SNPs only explained a minor proportion of the variation in plasma t-PA. On the other hand, another study showed that there is an independent association of the insertion allele of the I/D polymorphism in the TPA gene with nonfatal myocardial infarction [13]. This genetic variant could have some influence on fybrinolytic system not explained so far. Our study did not show any statistical significance between TPA Alu I/D genotypes and MS, age of onset or progression of the disease.

In conclusion, hereby we have presented the results that TPA DD/PAI-1 4G4G genotype combination might have a protective effect against developing MS.

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