

Short communication

Polymorphism 4G/5G in the plasminogen activator inhibitor-1 (PAI-1) gene is associated with IgE-mediated allergic diseases and asthma in the Czech population

Background: Plasminogen activator inhibitor type 1 (PAI-1) is a glycoprotein that belongs to the serine protease inhibitor superfamily and has an essential role in tissue remodeling after inflammation. Recently, a single base pair deletion/insertion (4G/5G) polymorphism of the PAI-1 gene has been associated with an increased risk of asthma in nuclear families from the UK. **Methods:** The present study was thus conducted to determine the association of this polymorphism with the development of IgE-mediated asthma and other allergic diseases in the Czech population. A case-control approach was used in our study. DNA taken from subjects with clinically manifested asthma and other allergic diseases ($n=207$) and from reference ethnically age-gender-matched unrelated subjects ($n=186$) was examined for base deletions/insertions in the PAI-1 gene.

Results: A significant association ($P=0.0035$) was observed between the PAI-1 promoter polymorphism and IgE-mediated allergic diseases altogether. Furthermore, the 4G allele frequency was also significantly higher in the asthmatic patients than in the control group ($P=0.0148$).

Conclusions: Our findings support the idea that the 4G allele of the 4G/5G polymorphism in the PAI-1 gene may be a risk factor for IgE-mediated asthma and allergic diseases.

D. Bučková, L. Izakovičová Hollá, J. Vácha

Department of Pathological Physiology, Medical Faculty, Masaryk University Brno, Czech Republic

Key words: allergic diseases, asthma, polymorphism, gene, PAI-1.

Accepted for publication 14 January 2002

Allergic diseases, most commonly occurring as rhinoconjunctivitis, asthma and atopic eczema/dermatitis syndrom (AEDS), are considered to be multifactorial inflammatory diseases involving complex interactions between genetic and environmental influences (1).

Every inflammatory process, including that in the course of bronchial asthma may disturb the balance in blood coagulation and the fibrinolysis system. Plasminogen activator inhibitor-1 (PAI-1) has a central role in the regulation of the fibrinolytic enzyme system and has an essential role in the airway remodeling of asthma (2). The PAI-1 gene has been assigned to chromosome 7 (q21.3-q22) and several polymorphisms in this gene including the deletion/insertion polymorphism (4G/5G) have been found to be significantly correlated with the plasma levels of PAI-1 (3) and differences in transcription activity *in vitro* (4). PAI-1, a member of the SERPIN family, is an acute-phase

reactant; its concentrations are increased by inflammatory stimuli such as interleukin(IL)-1 and tumour necrosis factor(TNF)- α . *In vitro* experiments have shown that the 4G allele produces six times more RNA than the 5G allele in response to IL-1 β (5). Individuals homozygous for the 4G allele have higher basal and inducible concentrations of PAI-1 than those with one or two copies of the 5G allele (5); the 5G allele is characterized by a lower transcriptional rate of the PAI-1 gene because of binding a transcriptional repressor protein which cannot be bound by the 4G allele (5, 6).

Recently, Cho et al. (7) reported a preferential transmission of the 4G allele to asthmatic children from the UK. Because gene pools, lifestyle and gene-environment interactions differ among populations, the risk should not be assumed to be similar in all populations for a given genetic trait. Therefore, ethnic differences in the allele frequency of the PAI-1 polymorphism and in the genetic association with disease may exist. The aim of our study was to test whether IgE-mediated allergic asthma or other allergic diseases are associated with the 4G/5G polymorphism in the PAI-1 gene also in our Czech population.

D. Bučková, Institute of Pathological Physiology, Medical Faculty, Masaryk University, Brno, Kom. namesti 2, 662 43, Czech Republic

Material and Methods

Characteristics of subjects

Caucasians of exclusively only Czech ethnicity ($n=422$) were included in this study. A case-control approach was used in our study. DNA taken from subjects with clinically manifested IgE-mediated asthma and/or other allergic diseases (IgE-mediated rhinitis, IgE-associated AEDS (the atopic eczema/dermatitis syndrome) (8), $n=207$), and from reference age-gender-selected unrelated healthy subjects ($n=186$) were examined for base substitutions in the PAI-1 gene (Table 1). The reference group selection was done in a random population sample with no personal and familial history of allergic diseases. The control group was selected in order to minimize possible effects of age and gender (about equal representation of persons as regards age composition and gender in the groups of both patients and controls), because it is important to compare the same aged population in association study.

Asthma was defined by using a questionnaire together with a physician's diagnosis of asthma. IgE-mediated allergic diseases was recognized by specific IgE (>0.35 kU/l) or a positive skin-prick test (SPT) to one or more allergens as described previously (9).

Project approval was granted by the local Ethics Committee at the Medical Faculty MU Brno and written informed consent for genetic analysis was obtained from all subjects.

Genotype identification

Genomic DNA was isolated from peripheral blood leukocytes by a standard method using the proteinase K digestion of cells.

Detection of the polymorphism

The PAI-1 4G/5G genotypes were determined by an allele-specific polymerase chain reaction (PCR) as described by Falk et al. (10). The amplified DNA fragments were separated by agarose gel electrophoresis and viewed after staining with ethidium bromide. The samples were genotyped and classified into one of the three possible genotypes 4G/4G, 5G/5G and 4G/5G.

Statistics

All statistical analyzes were performed using the programme package Statistica vs. 3.0 (StatSoft Inc Tulsa, USA). The χ^2 test and Fisher's exact test were performed to test for a comparison of differences in genotype or allele frequencies among groups. Odds ratio (OR), confidence intervals, and significance values were calculated.

Results

The characteristics of each group are shown in Table 1. As the two groups were age- and sex-matched, there were no significant differences in these variables.

The PAI-1 4G/5G genotype distributions and allele frequencies in the study groups are shown in Table 2. The genotype distributions were in Hardy-Weinberg equilibrium, both within each group and in the population as a whole.

While the frequencies of the 4G allele significantly exceeded the value found in the controls (0.508) both in the allergic diseases group (0.606, $P=0.0035$) and in the IgE-mediated allergic asthmatic subgroup (0.596, $P=0.0148$), the frequencies of genotypes differed significantly from the controls only in the allergic group (odds

Table 1. Characteristics of the studied subjects

Phenotype	Control group	Allergic group	Asthmatic subgroup
Number	186	207	159
Age (mean $\pm$ SD)	32.6 $\pm$ 10.4	28.8 $\pm$ 12.0	26.9 $\pm$ 12.8
Sex (M/F)	105/81	112/95	89/70

ratio: OR = 2.01, 95% CI: 1.22–3.31, $P=0.0153$), with a decreased frequency of the 5G/5G homozygotes in the group of allergic diseases.

In allergic subjects, there were no significant associations among the PAI-1 alleles and/or genotypes and several quantitative traits investigated in our study, including total serum IgE levels, selected specific IgE levels and skin test responses to aeroallergens (data not shown).

Discussion

In this study we examined the potential role of the PAI-1 deletion allele (4G) in the pathogenesis of IgE-mediated allergic diseases. Genome-wide screenings for susceptibilities to allergy have highlighted several candidate regions till this time. One such locus is at chromosome 7q, which contains the PAI-1 gene, where Xu et al. (11) have recently mapped a locus linked to IgE levels in Dutch families.

Using the case-control study design, we demonstrated an increased frequency of the 4G allele in allergic patients. Our study is not the first investigation of the PAI-1 gene polymorphism in asthmatic diseases. Previously, Cho et al. (2) found that the PAI-1 gene was induced in lung mast cells in asthmatic subjects. More recently, Cho and coworkers (7) have reported a significantly increased frequency of the 4G allele of the PAI-1 promoter polymorphism in an asthmatic population from the UK. The prevalence of the 4G allele in the Czech allergic patients is slightly lower than that reported in a previous UK study of asthmatic families, but the overall tendency of our results agrees with that obtained in the British population (7) and implies that a possible risk effect of the 4G/5G polymorphism of the PAI-1 gene is present also in the Czech population. In addition, contrary to previously published findings by

Table 2. Distribution of genotypes and allele frequencies of the 4G/5G PAI-1 polymorphism in controls and the IgE-mediated allergy group (IgE-mediated asthmatic subgroup)

	4G/4G (%)	4G/5G (%)	5G/5G (%)	4G	5G	P
Subjects						
Control group ($n=186$)	53 (28.5)	83 (44.6)	50 (26.9)	0.508	0.492	
Allergic group ($n=207$)	76 (36.7)	99 (47.8)	32 (15.5)	0.606	0.394	$P_a=0.0035$
Asthmatic subgroup ($n=159$)	57 (35.9)	75 (47.2)	27 (17.0)	0.596	0.404	$P_b=0.0148$

P_a value: the statistical significance for the comparison of allele frequencies between the control and IgE-mediated allergic groups (Fisher's exact test).

P_b value: the statistical significance for the comparison of allele frequencies between the control group and the subselect IgE-mediated asthmatic group (Fisher's exact test).

Xu et al., (11) we were unable to show any significant association between total serum IgE levels (and/or other associated quantitative traits, e.g., specific IgE levels, SPT positivity, etc.) and the PAI-1 polymorphism in chromosome 7q in the Czech population. Thus, there may be a significant ethnic difference in the genetic determination of total serum IgE levels. Our finding that a polymorphism, which influences the expression of an inhibitor of the fibrinolytic pathway, is significantly related to allergic diseases may seem surprising. However, there is evidence that the events of activating coagulation and inflammation are closely related and mutually interdependent and in primitive species, there is a single pathway for both coagulation and inflammation (12).

In conclusion, we have shown that Czech patients with IgE-mediated allergic diseases have a profile of the PAI-1 4G/5G polymorphism distinct from normal population; the deletion 4G variant may play as yet undefined role in the development of IgE-mediated

allergic asthma or other allergic diseases. This polymorphism should therefore be added to the current spectrum of genetic factors known to be involved in the pathogenesis of this multifactorial disease. However, as a difference in genetic background greatly influences allele frequencies of a disease-associated gene, further studies in different races might provide us with more information on the contribution of the PAI-1 in the etiopathogenesis of IgE-mediated allergic diseases including asthma.

Acknowledgments

The study was supported by project CEZ: 307/98: 141100002 'Molecular pathophysiology of multigenic diseases' provided by the Ministry of Education, Youth and Physical Training of the Czech Republic. We acknowledge Associate Professor V. Znojil for his statistical advice and Andrea Stejskalova for her excellent technical assistance.

References

- HOLGATE ST. Genetic and environmental interaction in allergy and asthma. *J Allergy Clin Immunol* 1999;**104**:1139–1146.
- CHO SH, TAM SW, DEMISSIE-SANDERS S, FILLER SA, OH CK. Production of plasminogen activator inhibitor-1 by human mast cells and its possible role in asthma. *J Immunol* 2000;**165**:3154–3161.
- MARGAGLIONE M, CAPPUCCI G, D'ADDEDA M, COLAIZZO D, GIULIANI N, VECCHIONE G, MASCOLO G, GRANDONE E, DI MINNO G. PAI-1 plasma levels in general population without clinical evidence of atherosclerosis: relation to environmental and genetic determination. *Atheroscler Thromb Vasc Biol* 1998;**18**:562–567.
- MATSUBARA Y, MURATA M, ISSHIKI I, WATANABE R, ZAMA T, WATANABE G, WATANABE K, IKEDA Y. Genotype frequency of plasminogen activator inhibitor-1 (PAI-1) 4G/5G polymorphism in healthy Japanese males and its relation to PAI-1 levels. *Int J Hematol* 1999;**69**:43–47.
- DAWSON SJ, WIMAN B, HAMSTEN A, GREEN F, HUMPHRIES S, HENNEY AM. The two allele sequence of a common polymorphism in the promoter of the plasminogen activator inhibitor (PAI-1) gene respond differently to interleukin-1 in HepG2 cells. *J Biol Chem* 1993;**268**:10739–10745.
- ERIKSSON P, KALLIN B, VAN'T HOOFT FM, BAVENHOLM P, HAMSTEN A. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. *Proc Natl Acad Sci USA* 1995;**92**:1851–1855.
- CHO SH, HALL IP, WHEATLEY A, DEWAR J, ABRAHA D, DEL MUNDO J, LEE H, OH CK. Possible role of the 4G/5G polymorphism of the plasminogen activator inhibitor 1 gene in the development of asthma. *J Allergy Clin Immunol* 2001;**108**:212–214.
- JOHANSSON SGO, HOURIHANE JOB, BOUSQUET J, BRUIJNZEEL-KOOMEN C, DREBORG S, HAAHTELA T, KOWALSKI ML, MYGIND A, RING J, VAN CAUWENBERGE P, VAN HAGE-HAMSTEN M, WÜTHRICH B. A revised nomenclature for allergy. *Allergy* 2001;**56**:813–824.
- IZAKOVICOVA HOLLA L, VASKU A, IZAKOVIC V, ZNOJIL V. The interaction of the polymorphisms in transporter of antigen peptides (TAP) and lymphotoxin α (LT- α) genes and atopic diseases in the Czech population. *Clin Exp Allergy* 2001;**31**:1418–1423.
- FALK G, ALMQVIST A, NORDENHEM A, SVENSSON H, WIMAN B. Allele specific PCR for detection of a sequence polymorphism in the promoter region of the plasminogen activator inhibitor-1 (PAI-1) gene. *Fibrinolysis* 1995;**9**:170–174.
- XU J, POSTMA DS, HOWARD TD, KOPPELMAN GH, ZHENG SL, STINE OC, BLEECKER ER, MEYERS DA. Major Genes Regulating Total Serum Immunoglobulin E Levels in Families with Asthma. *Am J Hum Genet* 2000;**67**:1163–1173.
- ESMON CT, TAYLOR FB, SNOW RT. Inflammation and coagulation: linked processes potentially regulated through a common pathway mediated by protein C. *Thromb Haemost* 1991;**66**:160–165.