

Possible role of the 4G/5G polymorphism of the plasminogen activator inhibitor 1 gene in the development of asthma

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Asthma represents a chronic inflammatory process of the airways. The plasminogen activator inhibitor 1 gene (*PAI1*) has an essential role in promoting fibrosis after inflammation. The tissue-type plasminogen activator and urokinase-type plasminogen activator convert plasminogen to plasmin, which enhances proteolytic degradation of the extracellular matrix (ECM). Among the inhibitors of plasminogen activators, PAI-1 is the most important in the process of lung fibrosis.¹ PAI-1-overexpressing mice underwent severe lung injury and deposition of ECM after bleomycin challenge, whereas PAI-1-deficient mice were protected against such fibrotic reactions.² These findings show that PAI-1 is closely associated with fibrosis and the accumulation of ECM after lung injury or inflammation. We previously reported that the *PAI1* gene is induced in lung mast cells in asthmatic subjects.³ These findings suggest that PAI-1 could contribute to tissue remodeling and subsequent fibrosis in asthma. The *PAI1* gene has variation in the promoter region on the basis of a single guanosine insertion-deletion (5G or 4G),^{4,5} and the *4G allele is correlated with increased plasma PAI-1 levels.^{5,6} In vitro experiments have initially shown that the *5G allele contains an additional binding site for a protein likely related to the nuclear factor κ B group of transcription factors, and this binding site is absent in the *4G allele.⁴ After stimulation with IL-1, HepG2 cells transfected with the *4G allele produce 6 times more PAI-1 mRNA than those with the *5G allele. These data suggest a functional role of the 4G/5G polymorphism in the response to cytokines, with the *4G allele being associated with enhanced gene expression. In the present study we demonstrate that the *4G allele is

Abbreviations used

ECM: Extracellular matrix
PAI: Plasminogen activator inhibitor
TDT: Transmission disequilibrium test

preferentially transmitted to asthmatic children. These data suggest a possible role of the *PAI1* gene and the 4G polymorphism in the pathophysiology of asthma.

METHODS

Characteristics of patients

To investigate the potential contribution of the PAI-1 biallelic single-nucleotide polymorphism to the risk of developing asthma, we studied siblings and parents from the Nottingham Asthma Family study. A total of 211 individuals from 51 families were genotyped. Families were identified on the basis of affected sib-pairs for asthma. The majority of sibling pairs were aged between 5 and 15 years. Asthma was defined by using a previously validated questionnaire together with a physician diagnosis of asthma.⁷ Atopy was defined as a positive skin prick test response to one or more common allergens (Der p 1, Fel d 1, and feathers). A positive response was considered to be a wheal of 3 mm or greater in size than that produced by the saline control. Histamine was used as a positive control.

Genomic DNA extraction

Blood was taken from each family member for genomic DNA extraction, which was performed with the QIAamp kit (Qiagen Inc, Valencia, Calif) as directed to obtain 3 to 12 μ g of DNA from 200 μ L of whole blood.

Determination of *PAI1* genotype

The *PAI1* *4G/5G genotype was analyzed with an allele-specific PCR modified from that of Falk et al,⁸ using an alternative forward primer (GTCTGGACACGTGGGGG for the *5G allele or GTCTGGACACGTGGGGA for the *4G allele) with a common reverse primer (TGCAGCCAGCCACGTGATTGTCTAG; designed to minimize primer-dimer formation) and a control upstream primer (AAGCTTTTACC ATGGTAACCCCTGGT). The PCR procedure included a hot-start initial step to avoid primer-dimer artifacts. The PCR mixture was subjected to 30-step cycles of 94°C (1 minute), 60°C (1 minute), and 72°C (1 minute). The PCR reaction was performed in a total volume of 25 μ L with 0.5 μ g of genomic DNA by using the Perkin-Elmer Cetus DNA thermal cycler (Perkin-Elmer, Corp [Applied Biosystems], Foster City, Calif). The reaction mixture contained 10 mmol/L TRIS-HCl (pH 8.0), 2.5 mmol/L MgCl₂, 200 μ mol/L deoxyribonucleoside triphosphates, and 25 pmol of each primer. For each PCR, 2.5 U of Taq polymerase (Promega, Madison, Wis) was used. Electrophoresis was performed in 2% agarose with

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1× TAE buffer. The gels were photographed after ethidium bromide staining (Fig 1). As a control of this PCR technique, PCR analysis was performed on DNA samples of known genotypes. Then more than 2 resulting PCR products for each genotype were sequenced to confirm the identity of the *4G and *5G alleles.

Statistical analysis

A transmission disequilibrium test (TDT) of the 4G/5G polymorphism was performed by using a sib-pair program to perform simple genetic analysis (SIB-PAIR version 0.99.3).⁹ The phenotypes studied were asthma and atopy. A *P* value of less than .05 was considered to be significant.

RESULTS

The distribution of the genotypes in the 102 children with asthma was determined as follows: 43 (42.2%) with *4G/4G, 50 (49.1%) with *4G/5G, and 9 (8.8%) with *5G/5G. The prevalence of the *4G allele was higher in this population than that in the normal populations of previous reports.² The results of the TDT between the *4G and *5G alleles in asthma and atopy in the families are shown in Table I. The *4G allele was preferentially transmitted to asthma-affected children ($\chi^2 = 6.6$, *P* = .0139). However, there was a lack of relationship between the *4G allele and the atopic children ($\chi^2 = 1.3$, *P* = .3123). These results suggest that the *4G allele of the *PAI1* gene may be associated with the development of asthma.

DISCUSSION

To investigate the potential contribution of polymorphism within the *PAI1* gene to the development of asthma, we studied nuclear families recruited from Nottingham, United Kingdom, on the basis of affected sib-pairs for asthma. Using the TDT approach, we demonstrated preferential transmission of the *4G allele to asthmatic children. However, preferential transmission of the *4G allele was not seen in the atopic subjects. The prevalence of the *4G allele in the Nottingham families is slightly higher than that reported in a previous United Kingdom study of healthy individuals,⁶ and the prevalence of the *4G allele was also higher than that reported in previous population-based studies of white populations.⁵ This difference could be explained either by differing prevalences of the *4G allele in various white populations or by the possibility that individuals carrying the *4G allele may have been preferentially included in this study because of an association of the allele with asthma. The demonstration of increased transmission of the *4G allele to asthmatic siblings is highly consistent with the observed functional effects of this allele in vitro⁴ and with the observation that there is an increased level of PAI-1 in the plasma of individuals carrying the *4G allele because of increased transcriptional activity of the gene.⁵ There are some difficulties in correlating the plasma level of PAI-1 and the 4G/5G polymorphism in asthma because treatment with steroids has been shown to increase PAI-1 levels.¹⁰ One would predict that in addition to potentially contributing to the development of asthma per se, this

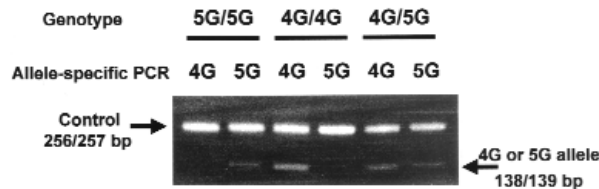


FIG 1. Gel patterns demonstrating the *4G and *5G alleles in the promoter region of the *PAI1* gene. PCR products with a forward primer for the *4G allele or the *5G allele and a control upstream primer are indicated by *4G or *5G, respectively. The first 2 lanes (patient A) represent the *5G/5G genotype, the second 2 lanes (patient B) represent the *4G/4G genotype, and the last 2 lanes (patient C) represent the *4G/5G genotype.

TABLE I. Transmission disequilibrium test for the *4G and *5G alleles in asthma and atopy

	Transmitted	Not transmitted	χ^2	<i>P</i> value
Asthma				
4G	44	23	6.6	.0139
5G	23	44		
Atopy				
4G	28	20	1.3	.3123
5G	20	28		

polymorphism might be important in determining the severity of disease and whether it persists into adulthood.

In summary, this study suggests that increased synthesis of PAI-1 may predispose to the development of asthma and contribute to the airway remodeling that occurs in this disease. If the pathophysiology of asthma is indeed associated with a defective fibrinolytic system caused by the inactivation of tissue-type plasminogen activator or urokinase-type plasminogen activator by PAI-1, then therapeutic interventions designed to enhance fibrinolysis, such as administration of plasminogen activators¹¹ or inhibitors of PAI-1,¹² may inhibit the development of asthma or reduce the chronic airway remodeling that occurs in the disease.

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