

# Plasminogen activator inhibitor-1 gene 4G/5G polymorphism in Turkish children with asthma and allergic rhinitis

Ozlem Yilmaz Ozbek, M.D.,\* F. Belgin Ataç, Ph.D.,# Ersin Ogus, Ph.D.,§ and Namik Ozbek, M.D.¶

## ABSTRACT

Plasminogen activator inhibitor (PAI-1) has an essential role in tissue remodeling after inflammation. Recent literature revealed only one study evaluating PAI-1 4G/5G gene polymorphism in children with asthma and none in children with allergic rhinitis. We aimed to investigate distribution of PAI-1 4G/5G polymorphism in a group of Turkish children with asthma and allergic rhinitis and compare these findings with those obtained in normal peers. Patients with physician-diagnosed asthma ( $n = 106$ ) and allergic rhinitis ( $n = 99$ ) and 83 healthy peers were included in this study. We evaluated PAI-1 4G/5G polymorphism genotype as well as the possible association between PAI-1 4G/5G polymorphism and pulmonary function tests, serum total immunoglobulin E (IgE), total eosinophil count, and skin-prick test positivity in our study. The prevalence of the 4G allele significantly exceeded the values found in the controls both in patients with asthma ( $p = 0.001$ ) and in patients with allergic rhinitis ( $p = 0.002$ ). Interestingly, comparison of asthmatic patients revealed that mean baseline percent forced expiratory volume in 1 second and forced vital capacity were significantly higher in patients who bear 5G/5G genotype than in those who have 4G/4G or 4G/5G genotypes. No statistically significant relationship were found between PAI-1 polymorphism and total serum IgE levels, total eosinophil count, or selected skin test responses to aeroallergens. Our study suggests that Turkish children with asthma or allergic rhinitis have a higher prevalence of PAI-1 4G allele compared with their healthy peers.

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**Key words:** Allergic rhinitis, asthma, children, eosinophilia, gene polymorphism, PAI-1 4G/5G polymorphism, pathophysiology, pulmonary function test, skin prick test, total IgE

Asthma and allergic rhinitis are deemed to be multifactorial inflammatory diseases. It is well known that disturbance of the balance among the blood coagulation and fibrinolysis systems may occur in every inflammatory process.<sup>1</sup> Plasminogen activator inhibitor 1 (PAI-1) is the main inhibitor of the fibrinolytic system not only in plasma, but also in the alveolar space. As an acute-phase reactant, PAI-1 concentrations could be increased by inflammatory stimuli. This protein may take place in the process of lung fibrosis.<sup>2</sup> Recent studies revealed a close association between overexpression of PAI-1 and accumulation of extracellular matrix (ECM) and fibrosis after lung injury or inflammation in mice.<sup>3</sup> Therefore, it is suggested that increased levels of PAI-1 could contribute to tissue remodeling and subsequent fibrosis in asthma.<sup>2–4</sup> Vascular endothelial growth factor (VEGF) is known to be

involved in the regulation of ECM degradation by increasing the expression of both plasminogen activators and PAI-1.<sup>5–7</sup> PAI-1 blocks the proteolytic activity of plasminogen activators and promotes ECM deposition and has been reported to play an essential role in tissue remodeling and might contribute to the development of chronic asthma.<sup>8</sup> Chung *et al.*<sup>9</sup> showed increased plasma levels of PAI-1 and VEGF in young children who had repeated wheeze, which were even higher in the children with more frequent relapses, suggesting that overproduction of PAI-1 and VEGF is associated with airway inflammation in these patients and might contribute to the later development of asthma. The plasma levels of circulating PAI-1 have been shown to be controlled at genome level, and a 4G/5G polymorphism at position –675 in the promoter region has been described. *In vitro* studies have shown that in response to inflammatory stimuli, 4G allele produces six times more PAI-1 mRNA than the 5G allele in HepG2 cell line.<sup>10</sup> In addition, plasma PAI-1 concentrations were higher in patients with asthma who had 4G allele compared with those who had 5G allele.<sup>11</sup> Recent literature revealed only one study conducted in children concerning the association between PAI-1 4G/5G polymorphism and asthma.<sup>12</sup> However, there were no studies in children about the association between PAI-1 genotype and allergic rhini-

From the Departments of \*Pediatric Allergy, #Medical Biology and Genetics, §Biostatistics, and ¶Pediatric Hematology, Baskent University Faculty of Medicine, Ankara, Turkey

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Address correspondence and reprint requests to Ozlem Yilmaz Ozbek, M.D., Department of Pediatrics, Baskent University, 6. Cadde No. 70/1, 06490 Bahcelievler, Ankara, Turkey

E-mail address: ozlemozbek@superonline.com

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tis. In this study, we aimed to investigate distribution of PAI-1 4G/5G polymorphism in a group of Turkish children with asthma and allergic rhinitis and compare these findings with those obtained in normal peers. In addition, the possible association between PAI-1 4G/5G polymorphism and lung function parameters, serum total immunoglobulin E (IgE), total eosinophil count, or skin-prick test (SPT) positivity was evaluated in children with asthma and allergic rhinitis.

## METHODS

Patients with physician-diagnosed asthma ( $n = 106$ ; 64 male and 42 female patients) and allergic rhinitis ( $n = 99$ ; 57 male and 42 female patients) were recruited from our Pediatric Allergy Department. A case-control approach was used in our study. Patients were eligible for the study if they were 6–16 years of age. Diagnosis of asthma was made according to the American Thoracic Society criteria,<sup>13</sup> *i.e.*, a history of recurrent cough, wheezing, and dyspnea on exposure to upper respiratory tract infections, irritants, and/or airborne allergens, together with a demonstrable reversible airflow limitation and/or increased airway responsiveness to methacholine.<sup>14,15</sup> During the subsequent 1-year follow-up period, these patients fulfilled Global Initiative for Asthma criteria for mild–moderate persistent asthma.<sup>16</sup> We treated our patients according to the guidelines of the Global Initiative for Asthma. Patients with asthma were included in the study if they had no attack for the last 2 months. Patients who had upper or lower respiratory tract infections for the last 1 month were excluded. Children with other chronic lung diseases and atopic dermatitis were not included in the analysis. At the time of inclusion in the clinical trial, a standard file included a detailed entry questionnaire including age at the onset of disease, duration, and severity of respiratory symptoms; trigger for symptoms; presence of other associated atopic diseases; type and dose of previous medical treatment; family history; and home conditions. This questionnaire was filled out for every child diagnosed with asthma. These children were also objectively assessed by physical examination, SPTs, and spirometry. All data in the patient files were regularly entered into the database program.

Allergic rhinitis was recognized by the following clinical manifestations: (1) a runny, itchy, congested nose, and sneezing attacks without having a cold for at least 2 years; (2) no previous symptoms of asthma ever; and (3) positive SPT to one or more aeroallergens. A total of 83 control subjects (47 male and 36 female patients) with no history of bronchial asthma and allergic rhinitis or other respiratory symptoms and no personal or familial history of allergic diseases were selected as controls. All control children were healthy,

nonobese (children with a body mass index below the 95th percentile for age and sex), and unrelated who visited our pediatric clinic for routine physical examination. All participants underwent a structured interview and physical examination.

We obtained approval from the relevant ethics committees and obtained written informed consent for laboratory analysis from all subjects. We recorded the age, sex, weight, and height of every participant. Serum total IgE levels, pulmonary function tests (PFTs), SPTs, and PAI-1 4G/5G genotype were obtained from all patients. In the control group, only PAI-1 4G/5G genotype was evaluated. We did not perform SPTs and PFTs in the control group.

## Skin-Prick Test

SPTs were performed on the forearm by use of standard skin-prick method. Tests were performed with standardized allergen extracts against the following most common aeroallergens: house-dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), grass mix (*Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*, *Avana sativa*, *Festuca elatior*, *Agrostis vulgaris*, *Holcus latanus*, and *Bromus*), weed mix (*Artemisia vulgaris*, *Plantago lanceolata*, and *Chenopodium album*), cereal mix (*Hordeum vulgare*, *Triticum vulgare*, *Avena sativa*, and *Zea mays*), tree mix (*Acer pseudoplatanus*, *Aesculus hippocastanum*, *Platanus vulgaris*, *Robinia pseudoacacia*, and *Tilia platyphyllos*), mold mix (*Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium*), dogs, and cats (Stallergens, Fresnes, France). Histamine hydrochloride, 10 mg/mL, and allergen diluents were used as positive and negative controls, respectively. A mean wheal diameter of at least 3 mm greater than that of the negative control was considered positive. Any child with such a response to one or more allergens was defined as atopic.

## Pulmonary Function Tests

PFTs were performed according to the American Thoracic Society guidelines<sup>14</sup> with a daily-calibrated spirometer ( $V_{\max}$  Spectra 22 Sensormedics body plethysmograph; Sensormedics Corp, Yorba Linda, CA). The test results revealed forced vital capacity (FVC), forced expiratory volume in 1 second ( $FEV_1$ ), the  $FEV_1$ /FVC ratio, and forced expiratory flow at 25–75% of the FVC. While performing spirometry, a minimum of three flow-volume loop results within 10% of each other were recorded and the flow-volume loop with the highest  $FEV_1$  analyzed. Twenty minutes after the completion of the test, a bronchodilator agent (200  $\mu$ g of salbutamol; Ventolin; Glaxo Wellcome, Marly le Roi, France) was administered with a volumatic spacer device (Glaxo Wellcome, Zeist, The Netherlands), and a PFT was performed. The subjects were diagnosed as

Table 1 Plasminogen activator inhibitor 1 genotype distribution and ages of patients and controls

| Genotype | Asthma     |            |            | Allergic Rhinitis |            |            | Healthy Controls |
|----------|------------|------------|------------|-------------------|------------|------------|------------------|
|          | Perennial  | Seasonal   | Total      | Perennial         | Seasonal   | Total      |                  |
| 4G/4G    |            |            |            |                   |            |            |                  |
| Number   | 12         | 30         | 44         | 9                 | 36         | 43         | 15               |
| %        | 40         | 39.5       | 41.5*      | 41                | 46.8       | 43.4**     | 18.3             |
| Mean age | 10.3 ± 3.1 | 10.1 ± 2.9 | 9.88 ± 2.8 | 12.2 ± 3.5        | 11.7 ± 3.2 | 11.8 ± 3.2 | 9.93 ± 2.7       |
| 4G/5G    |            |            |            |                   |            |            |                  |
| Number   | 13         | 27         | 39         | 8                 | 23         | 32         | 41               |
| %        | 43.3       | 35.5       | 36.8       | 36.3              | 29.8       | 32.3       | 50               |
| Mean age | 9.6 ± 3.4  | 9.0 ± 1.7  | 8.97 ± 2.2 | 11.8 ± 3.5        | 12.8 ± 2.5 | 12.7 ± 2.6 | 11.3 ± 3.3       |
| 5G/5G    |            |            |            |                   |            |            |                  |
| Number   | 5          | 19         | 23         | 5                 | 18         | 24         | 27               |
| %        | 16.7       | 25         | 21.7       | 22.7              | 23.4       | 24.2       | 31.7             |
| Mean age | 8.3 ± 1.6  | 10.1 ± 3.6 | 9.52 ± 3.3 | 9.8 ± 2.6         | 11.2 ± 3.4 | 10.8 ± 3.3 | 10.8 ± 3.6       |
| Total    |            |            |            |                   |            |            |                  |
| Number   | 30         | 76         | 106        | 22                | 77         | 99         | 83               |
| Mean age | 9.60 ± 3.1 | 9.74 ± 2.7 | 9.47 ± 2.8 | 11.9 ± 3.4        | 11.9 ± 3.1 | 10.9 ± 3.1 | 10.8 ± 3.3       |

\* $p = 0.001$  when compared with control group; \*\* $p = 0.002$  when compared with control group.

having a positive response to bronchodilator if the FEV<sub>1</sub> increased by at least 12% after treatment with a bronchodilator agent. All results were corrected for temperature, pressure, and saturated conditions. Treatment with a short-acting or long-acting bronchodilator was withheld for at least 8 hours before the PFT was performed.

### PAI-1 4G/5G Genotyping

Genomic DNA was prepared from leukocyte pellets by sodium dodecylsulfate lysis, ammonium acetate extraction, and ethanol precipitation. The primers and polymerase chain reaction (PCR) conditions were performed as previously described.<sup>17</sup> A 99-bp PCR product was digested with Bsl I. The uncut product (99 bp) shows the presence of 4G allele. If the PCR product was cut into two fragments as 77 and 22 bp, it revealed the 5G allele.

### Statistics

Statistical calculations were performed using SPSS software (Statistical Package for the Social Sciences, Version 11.5; SSPS, Inc., Chicago, IL). Results for all measurements are expressed as mean ± standard deviation (SD) and median. The Kolmogorov-Smirnow test was used to formally evaluate the normality of distributions. The  $\chi^2$ -test was performed to test for a comparison of differences in genotype frequencies among groups. Kruskal-Wallis and Dunn tests were evaluated to compare the groups. A value of  $p = 0.05$  or less was considered statistically significant.

### RESULTS

The mean ages of the patients with asthma, allergic rhinitis, and healthy controls were  $9.47 \pm 2.79$  years,  $10.9 \pm 3.11$  years, and  $10.8 \pm 3.3$  years, respectively. Because the three groups were age and sex matched, there were no significant differences in these variables. The frequencies of the genotypes in the 106 children with asthma were as follows: 44 (41.5%) with 4G/4G, 39 (36.8%) with 4G/5G, and 23 (21.7%) with 5G/5G. The same distribution in 99 children with allergic rhinitis was as follows: 43 (43.4%) with 4G/4G, 32 (32.3%) with 4G/5G, and 24 (24.2%) with 5G/5G (Table 1). The prevalence of the 4G allele significantly exceeded the values found in the controls both in patients with asthma ( $p = 0.001$ ) and in patients with allergic rhinitis ( $p = 0.002$ ). In addition, the frequencies of genotypes differed significantly from controls in patients with asthma and allergic rhinitis, with a decreased frequency of the 5G/5G genotype in patients compared with controls. Interestingly, comparison of subgroups within asthmatic patients revealed that mean baseline percent FEV<sub>1</sub> and FVC were significantly higher in patients who bear 5G/5G genotype than those who have 4G/4G or 4G/5G genotype (Table 2). However, PFT results were not different in patients with allergic rhinitis when we compared them according to their genotypes.

We divided the patients with allergic rhinitis or asthma into two subgroups concerning the pattern of sensitization to individual allergens: those who have seasonal or perennial disease. Twenty-four percent of patients with allergic rhinitis and 28% of patients with

**Table 2 Characteristics of asthmatic children in relation to plasminogen activator inhibitor 1 4G/5G polymorphism**

|                                | 4G/4G             | 4G/5G             | 5G/5G             | 4G/4G vs 4G/5G<br>( <i>p</i> -Value) | 4G/4G vs 5G/5G<br>( <i>p</i> -Value) |
|--------------------------------|-------------------|-------------------|-------------------|--------------------------------------|--------------------------------------|
| Total IgE (UI/mL)              |                   |                   |                   |                                      |                                      |
| Mean $\pm$ SD                  | 211.0 $\pm$ 363.7 | 257.7 $\pm$ 709.4 | 253.4 $\pm$ 404.1 | >0.05                                | >0.05                                |
| Median                         | 57.60             | 67.05             | 66.35             |                                      |                                      |
| Serum eosinophil (%)           |                   |                   |                   |                                      |                                      |
| Mean $\pm$ SD                  | 3.49 $\pm$ 3.10   | 3.88 $\pm$ 3.54   | 3.06 $\pm$ 2.47   | >0.05                                | >0.05                                |
| Median                         | 2.59              | 2.98              |                   |                                      |                                      |
| FVC (% predicted)              |                   |                   |                   |                                      |                                      |
| Mean $\pm$ SD                  | 82.5 $\pm$ 11.3   | 87.7 $\pm$ 10.8   | 89.8 $\pm$ 13.1   | <0.05                                | <0.001                               |
| Median                         | 82.00             | 88.00             | 88.00             |                                      |                                      |
| FEV <sub>1</sub> (% predicted) |                   |                   |                   |                                      |                                      |
| Mean $\pm$ SD                  | 91.0 $\pm$ 11.9   | 94.0 $\pm$ 11.9   | 101.3 $\pm$ 15.9  | <0.05                                | <0.001                               |
| median                         | 91.00             | 94.00             | 99.00             |                                      |                                      |
| FEF <sub>25-75</sub> (%)       |                   |                   |                   |                                      |                                      |
| Mean $\pm$ SD                  | 92.1 $\pm$ 23.4   | 94.4 $\pm$ 28.1   | 104.5 $\pm$ 28.1  | >0.05                                | >0.05                                |
| Median                         | 88.5              | 91.00             | 100.00            |                                      |                                      |

FEF<sub>25-75</sub> = forced expiratory flow at 25–75%; FEV<sub>1</sub> = forced expiratory volume in 1 s; FVC = forced vital capacity.

asthma were sensitive to perennial allergens. Mean ages and numbers of patients who had perennial or seasonal allergic disease were not different both in allergic rhinitis and in asthma groups ( $p > 0.05$ ). We also divided perennial and seasonal allergic rhinitis and asthma patients according to their PAI-1 genotype. There were also no differences between the numbers and ages of each specific genotypic subgroup (Table 1). In asthma and allergic rhinitis subjects, there were no significant associations among the PAI-1 alleles and/or genotypes and several quantitative features investigated in our study, including serum total IgE levels, total eosinophil count, and selected skin test responses to aeroallergens (data not shown).

## DISCUSSION

It is well known that homozygotes for the 4G allele have higher basal and inducible concentrations of plasma PAI-1 (higher activity of PAI-1) compared with individuals bearing one or two copies of 5G allele.<sup>10,18</sup> In a recent study, Sejima *et al.*<sup>19</sup> reported that expression of PAI-1 mRNA was significantly higher in allergic nasal mucosa than in normal mucosa. In addition, PAI-1 mRNA was detected in serous cells and epithelium in patients with allergic rhinitis in contrast to that in normal nasal tissues. These observations suggest that increased PAI-1 levels caused by the 4G allele may play an important role in allergic rhinitis. Buckova *et al.*<sup>20</sup> found similar results in a group of adult Czech patients with allergic rhinitis and atopic eczema. However, they included all of the patients in one group and compared them with normal persons, which makes

individual conclusions about allergic rhinitis unlikely. For the first time in children, we were able to show that the presence of PAI-1 4G allele was higher in patients with allergic rhinitis than in healthy children. Using the case-control study design, we showed an increased frequency of the 4G allele also in children with asthma. Prevalence and ages of the 4G/4G genotype were similar in our asthmatic and rhinitis groups. A recent longitudinal population-based study disclosed that rhinitis, even in the absence of atopy, was a powerful predictor of adult-onset asthma.<sup>21</sup> The same distribution of PAI-1 alleles in our patients with rhinitis and asthma may suggest that the same pathogenesis could be responsible for both diseases. Therefore, we think our patients with rhinitis may develop asthma during adulthood.

Previously, four studies in three different ethnic groups have been reported with a significant increase in the percent of frequencies of the PAI-1 4G allele in children<sup>12</sup> and adults<sup>20,22,23</sup> with asthma. In one of these studies, the 4G allele was more frequently encountered in house-dust mite-allergic adult asthmatic patients.<sup>23</sup> Our results agree with that obtained in British children<sup>12</sup> and Czech<sup>20</sup> and Polish<sup>22,23</sup> adult populations (Table 3). Consequently, these studies, conducted in four different ethnic groups including our study, suggest an increased frequency of PAI-1 4G allele in patients with asthma. Because the numbers of patients in all of these studies are relatively small, additional studies in different ethnic groups might provide us with more information on the contribution of

**Table 3 Comparison of percent frequencies of plasminogen activator inhibitor 1 genotypes in the literature and our study results in asthmatic patients**

|         | <b>Our Study<br/>(n = 106)</b> | <b>Ref. 12<br/>(n = 102)</b> | <b>Ref. 20<br/>(n = 159)</b> | <b>Ref. 22<br/>(n = 127)</b> | <b>Ref. 23<br/>(n = 372)</b> |
|---------|--------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| 4G/4G % | 41.5                           | 42.2                         | 35.9                         | 48                           | 46.3                         |
| 4G/5G % | 36.8                           | 49.1                         | 47.2                         | 42                           | 41.4                         |
| 5G/5G % | 21.7                           | 8.8                          | 17                           | 10                           | 10.2                         |

PAI-1 genotype in the etiopathogenesis of asthma or allergic rhinitis.

In humans, the gene for PAI-1 is located on chromosome 7 (q21.3-q22), where a locus related to IgE levels has also been mapped.<sup>24</sup> This finding suggests a link between PAI-1 gene and atopic diseases. Our study was unable to show any significant association between total serum IgE levels, total eosinophil count, positive SPT, and the PAI-1 genotype in accordance with the study conducted in Czech adults.<sup>20</sup> However, opposite findings have been reported in the Polish studies<sup>22,23</sup> mentioned previously and in a study from the United States.<sup>24</sup> The mean total IgE levels were associated with PAI-1 genotype, being higher in 4G/4G homozygotes than in 5G/5G homozygotes in the Polish studies.<sup>22,23</sup> Furthermore, significant correlation between plasma PAI-1 concentrations and total IgE levels has been reported in the study from the United States.<sup>24</sup> In a recent study, a positive haplotypic association between the  $\beta$ -chain of the high-affinity receptor for IgE (FCER1- $\beta$ ) and PAI-1 genes has been suggested in asthma susceptibility in Japan adults with asthma.<sup>25</sup> On the other hand, when they compared these two variables with asthma susceptibility one at a time, they could not show a direct relationship between asthma and PAI-1 or FCER1- $\beta$  genotypes. Thus, there may be an ethnic difference in the genetic determination of total serum IgE levels. Pulmonary function, bronchial reactivity, and total IgE levels may also be influenced by medications in our patients such as leukotriene antagonists.<sup>26</sup> Also, different patterns of sensitization to individual allergens or other unknown genetic factors could be responsible of this finding.

Experimental studies indicated that PAI-1 could potentially influence both inflammatory response and tissue remodeling.<sup>3,27</sup> A recent study from our country showed that plasma levels of PAI-1 were statistically higher than those in controls during acute asthmatic attack before starting steroid therapy and after receiving 1 week of steroid therapy.<sup>28</sup> The authors concluded that elevated PAI-1 levels may also indicate the activated endothelium, enhancing airway remodeling in asthma.<sup>28</sup> The Polish study mentioned previously indirectly supported these observations showing a strong correlation between plasma PAI-Ag concentra-

tion and bronchial reactivity to histamine in asthmatic patients.<sup>22</sup> In this study, mean baseline FEV<sub>1</sub> was found significantly lower in patients homozygous for 4G allele than in patients homozygous for 5G allele. Our study also revealed that mean baseline FEV<sub>1</sub> and FVC were significantly lower in patients with 4G/4G genotype than in those with either 4G/5G or 5G/5G genotype. Although prevalence of the 4G/4G genotype was similar in the asthmatic and rhinitis groups, FEV<sub>1</sub> and FVC of patients with allergic rhinitis who had 4G/4G genotype were not lower than the rest of the patients in this group. One explanation may be the pattern of sensitization to individual allergens different in those groups. Another explanation may be associated with age-related differences in the development of IgE response and bronchial reactivity.

In conclusion, we have shown that prevalence of the PAI-1 4G allele was higher in Turkish children with asthma or allergic rhinitis compared with those in healthy children. Having the 4G allele, either in heterozygous or homozygous state, presently, may play an undefined role in the development of asthma or allergic rhinitis. Therapeutic interventions designed to enhance fibrinolysis such as plasminogen activators or inhibitors of PAI-1 may inhibit the development of airway remodeling.<sup>1</sup> We believe multicenter and multinational studies with larger patient groups are needed to reach a definite conclusion concerning effects of genetic background and interactions in the pathogenesis of asthma and allergic rhinitis.

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