ORIGINAL ARTICLE

Analysis of -675 4G/5G *SERPINE1* and C-159T *CD14* Polymorphisms in House Dust Mite-Allergic Asthma Patients

K Kowal,[1] A Bodzenta-Lukaszyk,[1] A Pampuch,[1,2] M Szmitkowski,[3] S Zukowski,[1] M Benedetta Donati,[2] L Iacoviello[2]

[1] Department of Allergology and Internal Medicine, Medical University of Bialystok, Poland
[2] Laboratory of Genetic and Environmental Epidemiology, Research Laboratories, "John Paul II" Center for High Technology Research and Education in Biomedical Sciences, Catholic University, Campobasso, Italy
[3] Department of Biochemical Diagnostics, Medical University of Bialystok, Poland

Abstract

Background: Experimental studies indicate that endogenous plasminogen activator inhibitor-1 (PAI-1, encoded by the gene *SERPINE1*) modulates the immune response to lipopolysaccharide (LPS). On the other hand, LPS induces PAI-1 secretion. Activation of individual cells by LPS is facilitated by CD14. The single nucleotide polymorphisms -675 4G/5G in *SERPINE1* and C-159T in *CD14* are major determinants of PAI-1 and CD14 expression, respectively.
Objective: To evaluate the frequency of the -675 4G/5G *SERPINE1* and C-159T *CD14* polymorphisms in house dust mite (HDM) allergic asthma patients.
Methods: The polymorphisms were evaluated in unrelated inhabitants of northeastern Poland, including 372 HDM-allergic asthmatic patients and 160 healthy nonatopic control subjects using polymerase chain reaction.
Results: Both the C allele of *CD14* and the 4G allele of *SERPINE1* were more frequently encountered in HDM-allergic asthmatic patients than in healthy control individuals. When the 5G/5G-TT/CT genotype was considered as a nonrisk genotype, all other genotypes were associated with asthma. The odds ratios ranged from 3.96 (95% confidence interval, 1.56-10.1) for the 5G/5G-CC genotype to 10.7 (95% confidence interval, 5.1-24.9) for the 4G/4G-CC genotype. Bronchial reactivity to histamine and total serum immunoglobulin (Ig) E levels were predominantly associated with the 4G/5G *SERPINE1* variants, while bronchial reactivity to *Dermatophagoides pteronyssinus* and serum concentrations of specific IgE against *D pteronyssinus* were predominantly associated with the C/T *CD14* variants. Patients with 4G/4G-CC genotype had the lowest forced expiratory volume in 1 second and the highest bronchial reactivity.
Conclusion: The *SERPINE1* and *CD14* polymorphisms studied here are associated with different aspects of bronchial reactivity and IgE response. Our results indicate that PAI-1 and CD14 may interact to affect susceptibility to allergic asthma.

Key words: Asthma. Genetics. Innate immunity. Plasminogen activator inhibitor.

Resumen

Antecedentes: Los estudios experimentales muestran que el inhibidor del activador de plasminógeno endógeno 1 (IAP-1, codificado por el gen *SERPINA1*) modula la respuesta inmunitaria frente al lipopolisacárido (LPS). Por otro lado, el LPS induce la secreción de IAP-1. El CD14 facilita la activación de las células individuales. Los polimorfismos de un solo nucleótido -675 4G / 5G en el *SERPINA1* y C-159T en el *CD14* son determinantes importantes de la expresión del IAP-1 y del CD14, respectivamente.
Objetivo: Evaluar la frecuencia de los polimorfismos-675 4G / 5G en el *SERPINA1* y C-159T en el *CD14*, en pacientes con asma por sensibilización a los ácaros del polvo doméstico (APD).
Métodos: Los polimorfismos se evaluaron en habitantes del nordeste de Polonia sin ninguna relación familiar. Se incluyeron 372 pacientes asmáticos alérgicos a los APD y 160 sujetos control, no atópicos, sanos, y se utilizó la reacción en cadena de la polimerasa.
Resultados: Se observó que tanto el alelo C del *CD14*, como el alelo 4G de la *SERPINA1* aparecían con más frecuencia en pacientes asmáticos alérgicos a los APD, que en los individuos control sanos. Cuando el genotipo 5G / 5G-TT / CT se consideró un genotipo sin riesgo, el resto de genotipos se relacionaron con el asma. Los cocientes de posibilidades oscilaron de 3,96 (95 % intervalo de confianza, 1,56-10,1) para el genotipo 5G / 5G-CC hasta 10,7 (95 % intervalo de confianza, 5,1–24,9) para el genotipo 4G / 4G-CC. La reactividad bronquial a la

histamina y las concentraciones totales de inmunoglobulina (Ig) E sérica se asociaron principalmente con las variantes de la *SERPINA1* 4G/5G, mientras que la reactividad bronquial frente a *Dermatophagoides pteronyssinus* y las concentraciones de IgE específica frente a *D pteronyssinus* se relacionaron principalmente con las variantes C/T *CD14*. Los pacientes con el genotipo 4G / 4G-CC presentaron el menor volumen espiratorio forzado en el primer segundo y la reactividad bronquial más elevada.
Conclusión: Los polimorfismos de la *SERPINA1* y del *CD14* estudiados están relacionados con diferentes aspectos de la reactividad bronquial y la respuesta de la IgE. Nuestros resultados indican que la IAP-1 y el CD14 pueden afectar la susceptibilidad del asma alérgica.

Palabras clave: Asma. Genética. Inmunidad innata. Inhibidor del activador de plasminógeno.

Introduction

Plasminogen activator inhibitor-1 (PAI-1, encoded by the gene *SERPINE1*) has recently been reported to be involved in the pathogenesis of asthma [1,2]. In humans, a single base deletion/insertion polymorphism (-675 4G/5G) in *SERPINE1* has been associated with asthma [3-7]. In steroid-naive house dust mite (HDM) allergic asthmatic patients, the presence of the 4G allele is associated with greater nonspecific bronchial hyperreactivity [5,6]. The association between the -675 4G/5G *SERPINE1* polymorphism and asthma depends on enhanced PAI-1 synthesis because it is totally abolished after adjustment for morning plasma PAI-1 concentration [5]. Further support for the role of PAI-1 in the development of asthma comes from experimental studies showing an association between local PAI-1 production in response to allergen challenge and irreversible airway remodeling [8-10].

Interestingly, an increasing body of evidence indicates that endogenous PAI-1 promotes a T helper (T_H) type 2 immune response and increased production of immunoglobulin (Ig) E [8,9]. Genome-wide screening for susceptibility to allergy demonstrates that, in Dutch families, the region of chromosome 7 (q21.3-q22) that contains the *SERPINE1* gene is linked to total serum IgE concentration [11]. Moreover, in HDM-allergic asthmatic patients, plasma PAI-1 levels correlate with total serum IgE concentration [5]. Recent experimental studies demonstrated mechanisms responsible for modulation of the immune response by PAI-1 [8,9]. Mice lacking *SERPINE1* that were sensitized to ovalbumin (OVA) and subsequently challenged with OVA had significantly reduced IgE production and nasal hyperresponsiveness when compared with sensitized and challenged wild-type mice [8]. Moreover, splenocytes derived from *SERPINE1* -/- mice produced less interleukin (IL) 4 and IL-5 when compared with wild-type mice [8]. Interestingly, endogenous PAI-1 dampens T_H1-type immune responses after lipopolysaccharide (LPS) challenge. In comparison with their wild-type counterparts, *SERPINE1* -/- mice have elevated plasma levels of interferon (IFN) γ after LPS challenge [9].

Environmental exposure to LPS has been shown to have a protective effect against the development of allergic diseases [12]. Activation of individual cells by LPS is facilitated by CD14 and functional polymorphic variants of the *CD14* gene modulate the response to LPS [13-16]. The most prevalent single nucleotide polymorphism in *CD14* , is the C-159T polymorphism, which is associated with changes in serum IgE level [15,17]. The genetic variants of the *CD14* promoter region affect both monocyte expression of CD14 and serum concentration of soluble CD14 [18,19]. Peripheral blood mononuclear cells (PBMC) from asthmatic children with the CC genotype produce more IL-4 upon concanavalin A stimulation than those from TT homozygotes [20]. Stimulation of PBMC with LPS results in greater secretion of IL-1ß and IL-10 in TT homozygotes than in CC homozygotes [20].

Interestingly, an association between the C-159T polymorphism in the *CD14* promoter and asthma or atopy has been demonstrated in some but not all populations studied [21-24]. Even within the same population, the association between the C-159T polymorphism and markers of atopy such as total serum IgE concentration or number of positive skin prick tests was demonstrated in one ethnic subgroup (non-Hispanic) but not in another (Hispanic) [19]. Therefore, in this study we decided to evaluate the most frequently encountered, functional single nucleotide polymorphisms of *SERPINE1* and *CD14* in a homogenous population of HDM-allergic asthmatic patients from northeastern Poland. In addition, we attempted to identify associations between the polymorphic variants of the studied genes and functional and immunologic parameters in those patients.

Methods

Five hundred thirty-two unrelated young white Polish adults, including 372 HDM-allergic asthmatic patients and 160 nonatopic healthy control subjects, participated in the study. All subjects were born and resided in northeastern Poland. There were 268 steroid-naïve mild asthmatics and 104 patients with moderate-to-severe asthma who were treated according to the guidelines of the Global Initiative for Asthma [25]. All asthmatic patients reported upper and lower respiratory symptoms upon exposure to house dust, had positive skin prick tests with *Dermatophagoides pteronyssinus* and elevated serum levels of *D pteronyssinus*-specific IgE (class 2 or greater). In steroid-naïve patients, significant bronchoconstriction after bronchial challenge with histamine and significant bronchoconstriction after bronchial challenge with *D pteronyssinus* were demonstrated. In all patients with moderate or severe asthma, significant (more than 12%) improvement in forced expiratory volume in 1 second (FEV_1) after inhalation of 200 µg of salbutamol was demonstrated. The allergen and histamine challenges were performed before any therapy was introduced. None of the patients had received allergen immunotherapy before the initial evaluation. The patients included in the study were nonsmokers, without any

systemic or metabolic disease. Patients suffering from upper or lower respiratory tract infections at the time of the study or within 3 months of the beginning of the study were not included. The control group consisted of healthy, nonatopic, nonsmoking volunteers who visited our clinic for routine annual physical evaluation. The study was approved by the local ethics committee and all participants provided written informed consent.

Skin Prick Tests

All subjects underwent skin prick testing with a panel of aeroallergens (Allergopharma, Reinbek, Germany), as described previously [26].

Bronchial Challenge

Histamine bronchial challenge was performed according to the method described by Ryan et al [27]. Briefly, all patients inhaled doubling concentrations of histamine starting from a concentration of 0.62 mg/mL. Aerosol was generated using a DeVilbis 646 nebulizer attached to a Rosenthal–French dosimeter. All subjects performed 5 inspiratory capacity breaths for a given histamine concentration. Forced expiratory maneuvers were performed 90 seconds after each fifth inhalation. The procedure was continued until either a reduction in FEV_1 of at least 20% was observed or a histamine concentration of 32 mg/mL was reached. Bronchial provocation with aqueous *D pteronyssinus* extract (Allergopharma, Reinbek, Germany) was performed according to the method described by Cockroft et al [28]. In brief, increasing doses of allergen (0.8, 4, 20, 100, 500 and 2500 biologically standardized units [BSU]) were administered using a DeVilbis 646 nebulizer attached to a Rosenthal–French dosimeter. Forced expiratory maneuvers were performed 15 minutes after inhalation of eachdose of the allergen extract. Allergen inhalations were continued until either at least a 20% reduction of FEV_1 was observed or a cumulative dose of 5000 BSU was reached. Then, FEV_1 was measured every 15 minutes during the first hour, every 60 minutes during the next 11 hours, and again after 24 hours.

Bronchial challenge with histamine or *D pteronyssinus* allergen extract was only performed in steroid-naïve HDM-allergic asthmatic patients.

Blood Samples

Citrate anticoagulated blood samples and serum samples were collected between 7 and 9 in the morning, as described previously [5,6]. Immediately after centrifugation at 3000 rpm for 20 minutes at 4°C, the supernatants were separated from the cell pellets and the samples were stored at –80°C until used for analysis.

DNA Extraction and Analysis of Polymorphisms

Genomic DNA was extracted from whole blood cell pellets using a DNA purification kit (Genomic DNA Purification System, Wizard, Promega Corporation, Madison, Wisconsin, USA). *SERPINE1* promoter 4G/5G genotypes were analyzed by allele-specific polymerase chain reaction (PCR), as described previously [5]. PCR products were separated by electrophoresis in a 3% agarose gel and visualized directly by ethidium bromide staining. The samples were genotyped and classified according to the 3 possible genotypes: 4G/4G, 4G/5G, and 5G/5G. The C(-159)T polymorphism was detected using a modification of the method described by Baldini et al [19]. A 497-base pair (bp) PCR product was generated using the following primers: 5'- GCC TCT GAC AGT TTA TGT AAT C – 3' and 5'-GTG CCA ACA GAT GAG GTT CAC – 3'. PCR was performed in a final volume of 25 µL containing 100 ng DNA, 100 ng/mL of each primer, 200 µM dNTP, 1.5 mM $MgCl_2$, 500 mM KCl, 100 mM Tris-HCl, 1% Triton X100, and 1 U Taq DNA polymerase. Samples were denatured for 4 minutes at 94°C and then cycled 30 times through the following steps: 30 seconds at 94°C, 30 seconds at 49°C, and 30 seconds at 72°C. Restriction enzyme digestion of the amplified fragments with 5 units of *Ava* II was followed by separation on a 2.5% agarose gel. The homozygous C allele appears as a single 497-bp band, the homozygous T allele as bands of 144 bp and 353 bp, and heterozygotes exhibit all 3 bands (144, 353, and 497 bp).

Immunologic Assays

Total serum IgE and specific serum IgE against *D pteronyssinus* were measured using the UniCap system (Pharmacia, Uppsala, Sweden). Plasma concentration of soluble CD14 was evaluated using a commercially available enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota, USA) according to the manufacturer's instructions.

Statistical Analysis

Hardy-Weinberg equilibrium was tested using the χ^2 test. Multiple continuous variables were compared using the Kruskall-Wallis test. Whenever significant results were obtained by the Kruskall-Wallis test, the Wilcoxon test was used to evaluate differences between individual subgroups. The χ^2 test was used to compare discrete variables. Data for continuous variables were expressed as medians with 95% confidence intervals (CI); a 2-tailed *P* value of less then .05 was chosen as the cutoff for statistical significance. Logistic regression analysis was used to estimate odds ratios (OR) for HDM-allergic asthma associated with different genotypes. Adjusted ORs were calculated using multivariate logistic regression analysis with adjustment for age and sex. All calculations were carried out using SAS version 9.1 statistical software (SAS Institute Inc, Cary, North Carolina, USA) [5].

Results

There was no difference in sex distribution and age between patients and healthy control subjects (Table 1). All genotypes were in Hardy-Weinberg equilibrium in both studied groups. The prevalence of the 4G allele was higher in patients than in controls (Table1). In the univariate analysis, the 4G/4G and 4G/5G genotypes of *SERPINE1* were significantly associated with asthma. When compared with the 5G/5G -675 genotype as a reference, the OR for the 4G/4G genotype was 3.99 (95%

Table 1. Patient Characteristics[a]

	Healthy Controls (n=160)	HDM-Allergic Asthmatic Patients (n=372)	Steroid Naïve HDM-Allergic Asthmatics (n=268)
Age, y	24 (23-26)	25 (23-26)[b]	24 (23-25)[c]
Sex, female/male	64/96	159/213[d]	110/158[e]
FEV_1, % predicted	107 (98-109)	82 (81-83)[f]	85.5 (84-87)[f]
Total IgE, kU/L	ND	372 (352-398)	358 (328-380)
DpIgE, kU/L	ND	16 (13-17.5)	15.1 (12-17)
PC_{20}, mg/mL	ND	–	3.2 (2.8-3.6)
PD_{20}, SBE	ND	–	949 (865-1045)
SERPINE1 4G allele, %	53	69[f]	65[g]
CD14 C allele, %	47	56[h]	53[i]
Soluble CD14, mg/mL	2.3 (1.98 – 2.64)	–	1.94 (1.85 – 1.98)[j]

Abbreviations: DpIgE, serum concentration of specific immunoglobulin E to *Dermatophagoides pteronyssinus*; FEV_1, forced expiratory volume in 1 second; HDM, house dust mite; IgE, immunoglobulin E; PC_{20}, concentration of a bronchoconstricting agent causing 20% fall in FEV_1; ND, not determined; PD_{20}, allergen dose causing 20% fall in FEV_1.

[a] Data are shown as median (95% confidence interval) unless otherwise indicated.

[b] $P = .23$ vs healthy controls

[c] $P = .44$ vs healthy controls

[d] $P = .63$ vs healthy controls

[e] $P = .92$ vs healthy controls

[f] $P < .001$ vs healthy controls

[g] $P = .02$ vs healthy controls

[h] $P = .012$ vs healthy controls

[i] $P = .27$ vs healthy controls

[i] $P < .005$ vs healthy controls

CI, 2.33 – 6.8), while for the 4G/5G genotype it was 2.6 (95% CI, 1.56 - 4.49). Adjustment for age and sex did not modify the association: OR = 4.11 (95% CI, 2.49 – 6.97) for the 4G/4G genotype and OR = 2.81 (95% CI, 1.74 – 4.63) for the 4G/5G genotype (Table 2). The prevalence of the C allele at position –159 in the promoter of *CD14* was greater in patients than in health control subjects (Table 1). Univariate analysis revealed a statistically significant association between asthma and the CC genotype but not the CT genotype. In comparison with the TT genotype as a reference, the OR for the CC genotype was 2.23 (95% CI, 1.35 – 3.67), while for the CT genotype it was 1.37 (95% CI, 0.87- 2.16). Adjustment for age and sex did not modify the associations: OR = 2.34 (95% CI, 1.43 – 3.71) for the CC and OR = 1.41 (95% CI, 0.91 – 2.24) for the CT genotype (Table 2). The association of the CC genotype, however, was totally abolished after adjustment for soluble CD14 concentration (OR = 0.94; 95% CI, 0.46 – 1.51). When the 5G/5G *SERPINE1* genotype coexisting with the T allele of *CD14* (5G/5G-TT/CT) was considered as a nonrisk genotype, all other genotypes were significantly associated with asthma, with ORs ranging from 3.96 (95% CI, 1.56-10.1) for the 5G/5G-CC genotype to 10.7 (95% CI, 5.1-24.9) for the 4G/4G-CC genotype (Table 2).

Analysis of the selected quantitative asthma traits revealed that both polymorphisms were associated with aspects of bronchial reactivity and immune response.

The plasma concentration of soluble CD14 was affected by the C-159T *CD14* polymorphism. The median plasma concentration was significantly lower in the CC homozygotes than in the CT heterozygotes or the TT homozygotes (Table 3). No difference in plasma concentration of soluble CD14 was found when the patient group was divided according to the -675 4G/5G *SERPINE1* polymorphism (Table 3).

Lung function including baseline spirometry and bronchial reactivity to histamine was only associated with the *SERPINE1* polymorphism. The median baseline FEV_1 was significantly greater in 5G homozygotes than in 4G/5G heterozygotes ($P < .01$) and in 4G homozygotes ($P < .01$) (Table 3). Similarly, 4G homozygotes displayed the greatest median bronchial reactivity to histamine, which was significantly higher than in 4G/5G heterozygotes ($P = .002$) and 5G homozygotes ($P < .0001$) (Table 3). However, no differences in baseline FEV_1 ($P = .81$) or bronchial reactivity to histamine ($P = .096$) were observed when the group of steroid-naïve patients was divided according to the C-159T *CD14* genotype. In contrast, when that group of patients was divided according to C-159T *CD14* genotype, statistically significant differences were observed in the median bronchial reactivity to *D pteronyssinus* ($P < .001$). CC homozygotes showed the greatest median bronchial reactivity to *D pteronyssinus*, which was significantly greater than in CT heterozygotes ($P < .001$) and TT homozygotes ($P < .001$) (Table 3). When bronchial reactivity to *D pteronyssinus* was evaluated

Table 2. Frequency of Individual Genotypes in House Dust Mite-Allergic Asthmatics and Healthy Control Subjects[a]

SERPINE1 Genotype	*CD14* Genotype	HDM-Allergic Asthmatics, %	Healthy Controls, %	OR[b]	95% CI
5G/5G	All	38 (10.2)	43 (26.8)	1	Reference
4G/5G	All	154 (41.4)	70 (43.8)	2.81	(1.74-4.63)
4G/4G	All	180 (46.3)	47 (29.4)	4.11	(2.49-6.97)
All	TT	79 (21.2)	45 (28.1)	1	Reference
All	CT	152 (40.9)	73 (45.6)	1.41	(0.91-2.24)
All	CC	141(37.9)	42 (26.3)	2.34	(1.43-3.71)
5G/5G	TT/CT	15 (4.0)	30 (18.8)	1	Reference
5G/5G	CC	23 (6.2)	13 (8.1)	3.96	(1.56-10.1)
4G/5G	TT/CT	104 (28.0)	54 (33.8)	4.01	(1.98 – 8.3)
4G/5G	CC	50 (13.4)	16 (10.0)	6.41	(2.8-14.5)
4G/4G	TT/CT	112 (30.1)	34 (21.2)	6.73	(3.2-13.8)
4G/4G	CC	68 (18.3)	13 (8.1)	10.7	(5.1-24.9)

Abbreviations: CI, confidence interval; HDM, house dust mite; OR, odds ratio.
[a] Data are shown as number of cases (%).
[b] Adjusted for sex and age.

Table 3. Association Between Individual -675 4G/5G *SERPINE1* or C-159T *CD14* Polymorphisms and Clinical or Immunologic Parameters in Steroid-Naïve House Dust Mite-Allergic Asthmatic Patients (n = 268)

CD14 *SERPINE1*	CC All	CT All	TT All	All 4G/4G	All 4G/5G	All 5G/5G
Duration, mo	9 (8.4-10)	11 (9-12)	10 (9-11)	9 (8-10.1)	10 (9-11)	10.5 (9-11)
FEV_1, %	89 (87-94)	91 (88-96)	93 (88-98)	87 (85-93)[e,f]	91 (88-96)[e]	96 (93-101)
Eos, cells/μL	260 (230-289)	240 (220-260)	250 (220-270)	250 (230-270)	250 (230-260)	240 (192-289)
PC_{20}, mg/mL	3.1 (1.85-3.6)	3.24 (2.6-3.8)	3.1 (2.7-3.7)	2.2 (2.0-2.6)[e,f]	3.3 (3.0-3.7)[e]	5.3 (4.9-6.6)
PD_{20}, SBE	481 (412-624)[b,c]	1061 (946-1269)[b]	1658 (1398-1940)	835 (663-900)[g]	1005 (884-1342)	1230 (766-1605)
Total serum IgE, kU/L	392 (356-444)[d]	356 (335-403)	350 (313-383)	469 (418-492)[e,f]	356 (337-378)[e]	206 (121-278)
DpIgE, kU/L	22 (18-24)[b,c]	13 (10,6-16.3)[b]	7.8 (5.5 – 9.6)	18 (11-16.3)[e]	13 (11-16.3)	11.3 (9-12.6)
Soluble CD14, mg/mL	1.71 (1.6-1.9)[b,c]	1,98 (1.89-2.12)	2.14 (1.89-2.49)	1.87 (1.76-1.98)	1.97 (1.75-2.12)	1.93 (1.45-2.19)

Abbreviations: DpIgE, serum concentration of specific immunoglobulin E to *Dermatophagoides pteronyssinus;* Eos, peripheral blood eosinophils; FEV_1, forced expiratory volume in 1 second; IgE, immunoglobulin E; PC_{20}, concentration of histamine causing 20% fall of FEV_1; PD_{20}, dose of *D pteronyssinus* allergen causing a 20% fall of FEV_1.
[a] Data are expressed as medians with 95% confidence intervals.
[b] $P < .001$ compared with TT homozygotes
[c] $P < .001$ compared with CT heterozygotes
[d] $P < .05$ compared with TT homozygotes
[e] $P < .01$ compared with 5G/5G homozygotes
[f] $P < .01$ compared with 4G/5G heterozygotes
[g] $P < .05$ compared with 5G/5G heterozygotes

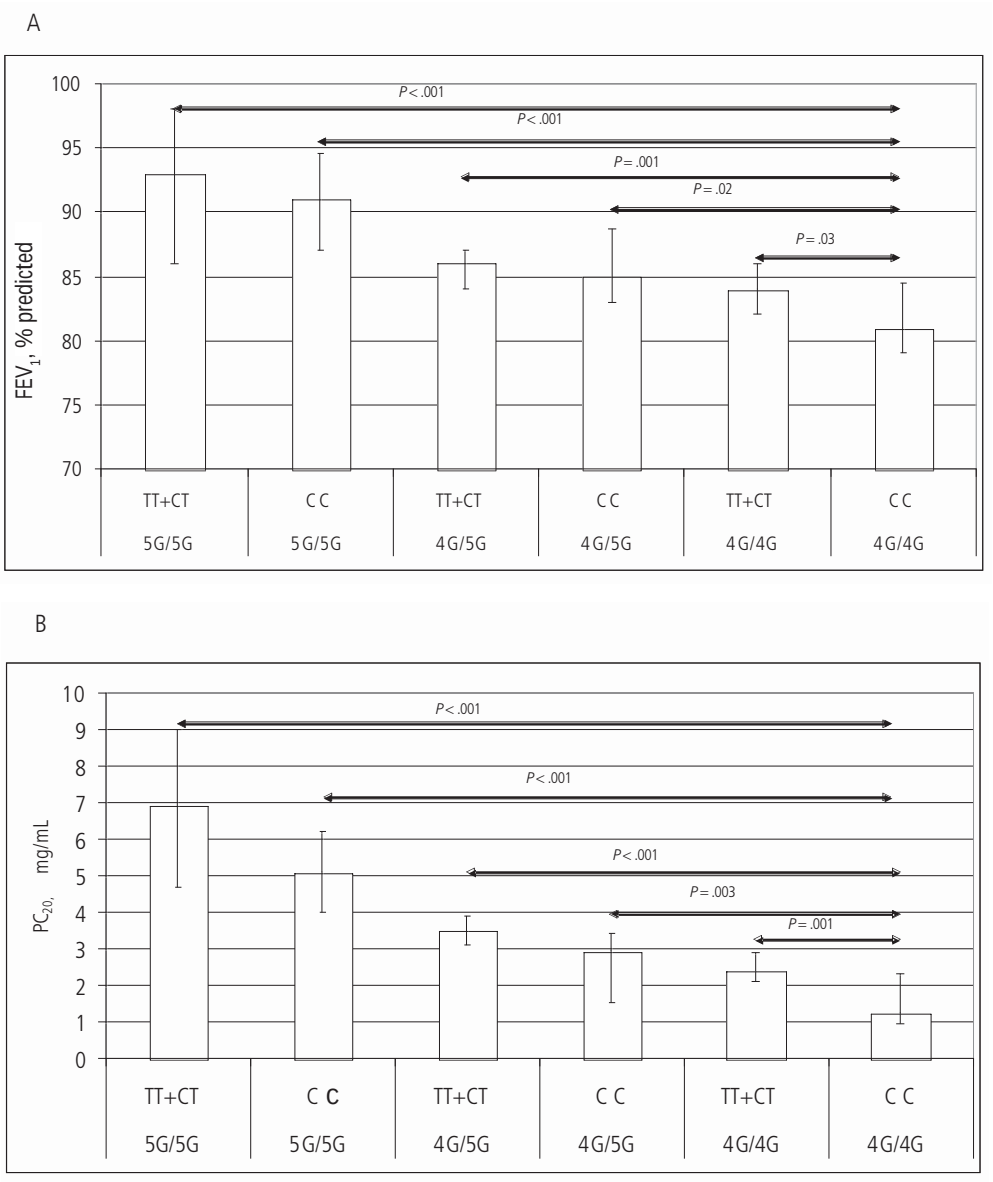

Figure. Lung function parameters in steroid-naive house dust mite-allergic asthmatics (n = 268) in relation to -675 4G/5G *SERPINE1* and C-159T *CD14* genotypes. Bars represent median forced expiratory volume in 1 second (FEV_1) (A) or bronchial reactivity to histamine expressed as median concentration of histamine causing a 20% fall in FEV_1 (PC_{20}) (B). Whiskers show 95% confidence intervals.

in the group of steroid-naïve patients divided according to 4G/5G -675 *SERPINE1* genotypes, a statistically significant difference was only seen between those carrying the 4G/4G and 5G/5G genotypes ($P = .022$) (Table 3). Analysis of lung function parameters in relation to combined genotypes revealed that those patients carrying both risk alleles of *SERPINE1* with both risk alleles of *CD14* (4G/4G-CC) had the lowest FEV_1 and the greatest bronchial reactivity to histamine (Figure). In contrast, the highest FEV_1 and the lowest bronchial reactivity to histamine were found in a subgroup of patients carrying the 5G/5G-CT/TT genotype (Figure).

Significant differences between median total serum IgE concentrations were seen when the group of steroid-naïve patients was divided according to 4G/5G-675 *SERPINE1* genotypes ($P < .0001$), but in subgroups divided according to C-159T *CD14* genotypes the difference was only significant between the TT homozygotes and the CC homozygotes ($P = .042$) (Table 3). However, significant differences in the concentration of serum specific IgE against *D pteronyssinus* were seen when steroid-naïve patients were stratified according to C-159T *CD14* genotypes ($P < .0001$), but when those patients were stratified according to 4G/5G -675 *SERPINE1* genotypes

the difference was significant only between the 4G/4G and the 5G/5G homozygotes (P = .02) (Table 3).

Discussion

The results of this study support previous findings demonstrating an association between functional polymorphisms in *CD14* or *SERPINE1* and asthma [29-31]. However, our study indicates that functional polymorphisms of *CD14* and *SERPINE1* are predominantly associated with different aspects of the asthmatic phenotype, eg, allergen-specific bronchial reactivity and nonspecific bronchial reactivity, respectively. Moreover, a possible genetic interaction between *CD14* and *SERPINE1* influencing susceptibility to HDM-allergic asthma may occur through an effect of those genes on different aspects of bronchial reactivity and IgE response.

We have previously reported that the -675 4G/5G *SERPINE1* promoter polymorphism is functional and the association between that polymorphism and asthma depends on enhanced PAI-1 synthesis [5,6]. The results of the current study indicate that the C-159T *CD14* polymorphism is also functional and its association with asthma depends on altered expression of *CD14*. Our results are consistent with other reports demonstrating associations between the -675 4G/5G *SERPINE1* polymorphism and allergic asthma in different populations [3-7] as well as an association between the C-159T *CD14* polymorphism and sensitization to HDM allergens [17,32]. Interestingly, sensitization to HDM allergens is recognized as an independent risk factor for asthma [25]; however, the association between the C-159T *CD14* polymorphism and asthma has been demonstrated in some but not all studies [14,21,24,27,32]. The preferential transmission of the C allele to children with atopic asthma was demonstrated in an Indian population [14] and an association between the T allele and lower asthma severity scores was found in a population from Barbados [24]. The results of those studies are in general agreement with our findings linking the C allele of *CD14* with increased risk of HDM-allergic asthma. Analysis of the frequency of the C allele in our patients provides further support for an association between that polymorphism and asthma severity. The increased frequency of the C allele was found in patients with moderate-to-severe asthma but not in mild asthmatics. The lack of an association between the C-159T polymorphism and asthma phenotype seen in other studies may be explained by heterogeneity of the study populations, including different proportions of atopic asthmatics, smoking history, and differences in the environmental exposure to LPS [21,27,33].

Asthma is a heterogenous disease and factors affecting asthma phenotype such as bronchial reactivity and serum IgE levels can be influenced by genetic and environmental factors including allergen exposure and medications used for asthma therapy. We therefore performed our study on a homogenous group of HDM-allergic asthmatic patients who were born and resided in northeastern Poland [5,6]. Moreover, analysis of associations between the studied polymorphisms and selected asthma traits was performed in a large subgroup of patients who had not received corticosteroids for treatment of asthma before the study. In our study, steroid-naïve patients with the CC genotype had significantly greater reactivity to *D pteronyssinus* challenge than those with the CT or TT genotypes, but no association between the C-159T *CD14* polymorphism and bronchial reactivity to histamine was found. Since this observation was made in mild asthmatic patients with a short duration of the disease, however, it is possible that the functional polymorphic variants of the *CD14* promoter may be associated with bronchoconstriction triggered by immunologic stimuli but that the association with airway remodeling and response to direct bronchoconstrictors is less stringent, at least in mild asthmatics in whom the disease appeared recently. Interestingly, in a population of asthmatic children, an association has been reported between the C-159T *CD14* polymorphism and increased severity of asthma exacerbations [31]. Those carrying the CC genotype were more likely to have moderate or severe asthma exacerbations. The strong association between the C-159T *CD14* polymorphism and the allergen-specific immune response may be explained by the character of natural exposure to house dust. It has been demonstrated that house dust samples contain both HDM allergen and LPS in different proportions [12,13]. Therefore, exposure to HDM allergen is inevitably associated with exposure to LPS [12,13,24]. Enhanced expression of CD14 such as that seen in subjects carrying the TT genotype leads to increased activation of cells involved in the immune response and increased secretion of proinflammatory mediators including tumor necrosis factor (TNF) α, IL-1ß, IL-12, and IFN-γ, skewing the immune response towards Th1 [9,20]. Proinflammatory cytokines released during tissue injury or allergen challenge stimulate secretion of PAI-1 and their effect is modulated by the -675 4G/5G *SERPINE1* promoter polymorphism [1,2,6]. Endogenous PAI-1 inhibits IL-12-dependent secretion of IFN-γ in response to challenge with LPS but also inhibits IL-12-independent IFN-γ release in response to staphylococcal enterotoxin B (SEB) [9]. The effect of PAI-1 on IgE synthesis, therefore, may be more promiscuous than that of CD14 because PAI-1 interferes not only with LPS-CD14-mediated cell activation but also with direct activation of T cells such as that seen after SEB stimulation. Furthermore, attenuated production of IL-4 and IL-5 in response to allergen challenge has been demonstrated in *SERPINE1* -/- mice compared with their wild-type counterparts [9]. Those observations may at least partially explain why in our study total serum IgE levels were associated more closely with the *SERPINE1* polymorphism than with the *CD14* polymorphism.

In summary, our results support previous observations concerning association of functional single nucleotide polymorphisms of *SERPINE1* and *CD14* with asthma and provide additional insights into the complexity of the relationship between genetic regulation of PAI-1 and CD14 expression and allergic asthma phenotype. Moreover, our results demonstrate that the -675 4G/5G *SERPINE1* polymorphism and the C-159T *CD14* polymorphism may cooperate in the development of asthmatic phenotype in HDM-allergic asthmatics by affecting different aspects of bronchial reactivity. We recognize that because of some specificities of HDM allergens our results may not be easily extrapolated to other asthma phenotypes and further studies are warranted to

determine whether our observations made in HDM-allergic asthmatics are also applicable to other asthma phenotypes.

Acknowledgments

This work was supported by a grant from the Medical University of Bialystok, Poland (research grant 3-35493) and from Telethon, Italy (contract No. E.1005). Partial support was also received from the European Union project IMMIDIET QLK1-CT-2000-00100 (L. I. and M. B. D).

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Manuscript received October 30, 2007; accepted for publication December 10, 2007.

Krzysztof Kowal

Department of Allergology and Internal Medicine
Medical University of Bialystok
Sklodowskiej-Curie 24a
15-276 Bialystok, Poland
E-mail: kowalkmd@amb.edu.pl

