ORIGINAL ARTICLE

The –2549 –2567 del18 Polymorphism in *VEGF* and Irreversible Bronchoconstriction in Asthmatics

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Abstract

Background: While the importance of vascular endothelial growth factor (VEGF) in the pathogenesis of several diseases (eg, neoplasms) has been proven, its role in asthma, especially in terms of the potential associations between genetic variants of VEGF and airway remodeling, has received relatively little attention.
Objectives: This study aimed to evaluate the possible connection between a genetic factor, ie, the polymorphism del/ins in the *VEGF* promoter region, and airway remodeling potential in asthmatics with and without irreversible bronchoconstriction.
Materials and Methods: The study population comprised 82 patients with asthma (of whom 42 had irreversible bronchoconstriction) and a group of 40 controls. DNA was isolated from peripheral blood leukocytes. Polymerase chain reaction was used to type the *VEGF* (18-bp deletion/insertion) gene polymorphism at loci –2549 –2567. Other factors (ie, smoking, disease duration) were also taken into consideration.
Results: The del/del genotype was found in 74.39% of patients with asthma (*P*=.031; OR=2.38), 80.95% of patients with irreversible bronchoconstriction (*P*=.012; OR=3.48), and 67.5% patients with reversible bronchoconstriction (*P*=.251; OR=1.70). The proportion of smokers to nonsmokers was higher (*P*=.032) and disease duration was longer (*P*=.041) in patients with irreversible bronchoconstriction than in those with reversible bronchoconstriction.
Conclusions: Our results showed that the risk of irreversible bronchoconstriction in asthmatics was associated with the presence of the del18 genotype at the –2549 –2567 position in the promoter region of the *VEGF* gene, as were disease duration and other factors such as smoking.

Key words: Vascular endothelial growth factor (*VEGF*). Gene polymorphisms. Asthma. Airway remodeling. Irreversible bronchoconstriction.

Resumen

Antecedentes: Aunque se ha demostrado la importancia del factor de crecimiento endotelial vascular (VEGF) en la patogénesis de varias enfermedades (p. ej. neoplasias), los datos relativos al asma son escasos, especialmente los relacionados con las posibles asociaciones entre las variantes genéticas de VEGF y la remodelación de las vías respiratorias.
Objetivos: En este estudio se propuso evaluar la posible relación entre un factor genético como el polimorfismo del/ins en la región promotora de VEGF y el potencial de remodelación de las vías aéreas en los asmáticos con y sin broncoconstricción irreversible.
Materiales y métodos: en el estudio participaron 82 pacientes con asma (42 pacientes con broncoconstricción irreversible) y un grupo de 40 controles. El ADN fue extraído de leucocitos de sangre periférica. Para la tipificación del polimorfismo del gen VEGF (delección / inserción de 18 pb) en loci -2549 -2567 se utilizó el método de reacción en cadena de la polimerasa (PCR). Se consideraron también otros factores (fumar, duración de la enfermedad).
Resultados: El genotipo del/del se encontró en el 74,39% de pacientes con asma (p = 0,031; OR = 2,38), el 80,95% de los pacientes con broncoconstricción irreversible (p = 0,012; OR = 3,48) y el 67,5% de los pacientes con broncoconstricción reversible (p = 0,251; OR = 1,70). La proporción de fumadores con respecto a los no fumadores fue mayor (p=0,032) y la duración de la enfermedad fue mayor en pacientes con broncoconstricción irreversible en comparación con aquellos con broncoconstricción reversible (p = 0,041).
Conclusiones: Nuestros resultados mostraron que la presencia del genotipo del18 en la posición -2549 -2567, en el promotor del gen VEGF, junto con la duración de la enfermedad y otros factores como fumar cigarrillos, se asocian con el riesgo de broncoconstricción irreversible en los individuos asmáticos.

Palabras clave: Factor de crecimiento del endotelio vascular. Polimorfismo génico. Asma. Remodelado de la vía respiratoria. Broncoconstricción irreversible.

Introduction

Vascular endothelial growth factor (VEGF) is an angiogenic factor produced by various types of cells under genetic control. The human *VEGF* gene has its chromosomal locus at 6p21.3 and contains a 14-kb coding region, which is divided into 8 exons separated by 7 introns. Expression of *VEGF* may be regulated by cytokines and growth factors (eg, PDGF, EGF, TGF-β), interleukins (eg, IL-1, IL-6), lipopolysaccharide, and oxygen deficiency in tissues (HIF) [1-4].

VEGF mediates pleiotropic functions. First, it is a prime regulator of endothelial cell proliferation. It also plays an important role in physiological angiogenesis, is involved in the TH2-mediated inflammatory response, and influences the proliferation of epithelial cells, fibroblasts, and tumor cells in carcinogenesis [5,6]. Overexpression of *VEGF* resulting in inappropriate *VEGF*-induced angiogenesis affects highly vascularized organs, including the lungs [7]. Expression of *VEGF* in bronchoalveolar lavage fluid is higher in asthmatics than in healthy controls, while overexpression in induced sputum and plasma is associated with disease severity [8,9]. Chetta at al [10] suggest that mucosal neovascularization is characteristic of chronic asthma and may lead to airway obstruction due to an inverse association between mucosal vascularization and airway diameter. Therefore, *VEGF* plays a special role in the pathogenesis of asthma and possible changes in the airway wall in asthmatics.

Asthma is a heterogeneous disease in which genetic and environmental factors contribute to the occurrence and severity of symptoms [11-14]. It is characterized by chronic airway inflammation, which, simultaneously with damage, activates repair processes, thus leading to *VEGF*-mediated neovascularization [15]. The polymorphic features of the promoter region of *VEGF* may influence the transcription potential of this gene. The meta-analysis performed within a genome-wide association study [16] revealed that the level of *VEGF* in blood may be interpreted in the light of various polymorphic features within the *VEGF* gene. Notably, the –2549 –2567 del18 polymorphism was found to be correlated with increased *VEGF* production, which in turn may contribute to airway remodeling with irreversible bronchoconstriction of differing severity [17-19].

In the literature, data on a possible association between this *VEGF* gene del/del variant and the risk of irreversible bronchoconstriction in patients with asthma are lacking. We address this gap in the present paper.

Materials and Methods

Population Study

The study sample included 122 individuals (80 females), of whom 82 were patients (54 females) diagnosed with asthma according to the Global Initiative for Asthma recommendations (GINA) and 40 were controls (26 females) without allergies or chronic pulmonary diseases. The presence of asthma and/or chronic obstructive pulmonary disease was determined on the basis of previous self-reported medical diagnoses, the GINA [11] and GOLD [20] guidelines, and the algorithm developed by Liebhart and Dor [21]. The degree of severity of asthma ranged from sporadic to severe persistent. The group of asthmatics was divided into 2 subgroups according to the presence of irreversible bronchoconstriction (42 patients; 25 females) and the absence of irreversible bronchoconstriction (40 patients; 29 females) based on bronchodilation testing. Genotyping of the *VEGF* polymorphism revealed the following genotypes: homozygous mutation insertion/insertion (ins/ins), heterozygous mutation deletion/insertion (del/ins), and no mutation, or the so-called wild genotype deletion/deletion (del/del, del18). The study was approved by the Ethics Committee at Wroclaw Medical University, Wroclaw, Poland (No. KB 68/2011). The demographic data and clinical profiles are shown in Table 1.

Bronchodilation Test

Pulmonary function and bronchodilation testing were performed in all groups examined. Forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) values were established using a Master Scope CT Spirometer (Erich Jaeger GmbH) and presented as a percentage of predicted values (% predicted). Each parameter was recorded 3 times, and the best score was used in further analysis. The bronchodilation test was performed after inhalation of 5 mg of salbutamol (SteriNeb Salamol, Teva Pharmaceuticals) administered with a jet nebulizer (Model 4650-U, Devilbiss). Reversible bronchoconstriction was defined as increases in postbronchodilator values (FEV_1 >120 mL and >12%).

Table 1. Demographic Data and Clinical Profiles

	No.	Female, No. (%)	Mean (SD) Age, y	Mean (SD) Disease Duration, y	Mean (SD) Baseline FVC, %	Mean (SD) Baseline FEV_1%	Mean (SD) Baseline FEV_1%/ FVC	Smoking Yes/No, %	Genotype del/del vs del/ins+ins/ins, No.
Asthma (RB)	40	29 (72.5%)	50.03 (13.65)	10.04 (8.28)	98.49 (13.89)	90.12 (17.25)	0.73 (0.10)	6/34 15%	27/13
Asthma (IB)	42	25 (59.52%)	53.71 (10.40)	21.94 (11.88)	72.21 (19.31)	56.05 (18.62)	0.61 (0.11)	15/27 35%	34/8
Controls	40	26 (65%)	47.95 (13.66)	–	101.36 (13.26)	104.81 (15.37)	0.82 (0.06)	11/29 27%	22/18

Abbreviations: del/ins, deletion/insertion; FEV_1%, forced expiratory volume in 1 second, % of predicted; FVC%, forced vital capacity, % of predicted; IB, irreversible bronchoconstriction; RB, reversible bronchoconstriction.

Isolation of DNA

DNA was isolated from peripheral blood lymphocytes using a DNA isolation kit (QiAmp DNA Blood Mini kit, Syngen Biotech) following the manufacturer's recommendations.

Genotyping of the VEGF Polymorphism

All asthmatics and controls were genotyped using polymerase chain reaction (PCR) following Lachheb et al [22] for the verification of the presence of the *VEGF* polymorphism, which comprises the addition or the loss of 18 base pairs (18 bp) within the promoter region at position –2549 –2567. The concentration of the isolated DNA and its purity were identified using a spectrophotometer (NanoDrop, Thermo Fisher Scientific). The PCR mixture was then prepared (total volume, 25 µL). This contained 100 ng of genomic DNA, 1 × Taq Buffer, 0.5 mmol/L of nucleotide, 3 pmol of suitable starter, and 0.5 units of Taq-DNA polymerase (Taq DNA Polimeraza E2500-02 – 5000u, EURx); the final $MgCl_2$ concentration was 4 mmol/L. The PCR comprised an initial denaturation step (95ºC for 15 min), then 35 cycles (95ºC for 30 s), primer annealing (54ºC for 30 s and 72ºC for 30 s), and a final extension step (72ºC for 10 min). The primer used for –2549 –2567 del18 was as follows: F, 5'-CCTGGAGCGTTTTGGTTAAA-3' and R, 5'-ATATAGGAAGCAGCTGGAA-3' (DNA primers, Polgen). The PCR products underwent electrophoresis in agarose gel stained with ethidium bromide. DNA in the form of strips was visible by fluorescence under a UV light transluminator. The fragment sizes were 234 bp when the 18-bp insertion was present and 216 bp when the 18-bp deletion was present.

Statistical Analysis

The data were analyzed using Statistica 10 for Windows. Within-group comparisons were made using the χ^2 test. The results are presented as the odds ratio (OR) with the 95%CI. Statistical significance was set at <.05.

Results

The general characteristics of the 82 patients with asthma and 40 controls are shown in Table 1. The groups were similar in terms of sex distribution (*P*=.167) and age (*P*=.169). Disease duration was longer in patients with irreversible bronchoconstriction (*P*=.041) than in the subgroup with reversible bronchoconstriction.

Among the asthmatics, 21 patients (25.6%) had a history of current smoking. In the subgroup of asthmatics with reversible bronchoconstriction, only 6 persons (15%) had a history of smoking; however, in the subgroup with irreversible bronchoconstriction, 15 patients (35.71%) had a positive smoking history. The proportion of smokers to nonsmokers was similar in asthmatics and in the control group (*P*=.824), although significantly higher in patients with irreversible bronchoconstriction than in those with reversible bronchoconstriction (*P*=.032) (Table 2). The mean smoking index in the group of asthmatics was relatively low (11.76 pack-years).

The del/del genotype was found in 61 patients (74.39%) with asthma: 34 (80.95%) with irreversible bronchoconstriction and 27 (67.5%) with reversible bronchoconstriction. In the control group, the del/del genotype was observed in 22 participants (55%) (Table 3).

There were statistically significant differences between asthmatics and controls in the distribution of the del/del genotype (*P*=.031; OR=2.38), as well as between the subgroup of patients with irreversible bronchoconstriction and individuals from the control group (*P*=.012; OR=3.48). No statistically significant differences were found in the distribution of the del/del genotype between controls and asthmatics with reversible bronchoconstriction (*P*=.251; OR=1.70) or between subgroups with reversible and irreversible bronchoconstriction (*P*=.163; OR=2.05) (Table 4).

Table 2. Comparison Between Participants With a Positive History of Smoking

	P Value[a]	OR (95%Cl)
Asthma vs controls	.824	0.91 (0.387-2.13)
Asthma with irreversible bronchoconstriction vs Asthma with reversible bronchoconstriction	.032	3.15 (0.97-10.62)

[a]χ^2 test.

Table 3. Distribution of del/del and ins/ins + ins/del Genotypes

VEGF Genotype	Asthmatics, No. (%)	Asthmatics (RB), No. (%)	Asthmatics (IB), No. (%)	Controls, No. (%)
del/del	61 (74.39%)	27 (67.5%)	34 (80.95%)	22 (55.0%)
ins/del + ins/ins	21 (25.61%)	13 (32.5%)	8 (19.05%)	18 (45.0%)

Abbreviations: IB, irreversible bronchoconstriction; RB, reversible bronchoconstriction; VEGF, vascular endothelial growth factor.

Table 4. Comparison Between Examined Groups in Terms of del/del Genotype

	P Value[a]	OR (95%Cl)
Asthmatics vs controls	.031	2.38 (1.00-5.69)
Asthmatics (RB) vs controls	.251	1.70 (0.62-4.67)
Asthmatics (IB) vs controls	.012	3.48 (1.17-10.6)
Asthmatics (IB) vs asthmatics (RB)	.163	2.05 (0.67-6.39)

Abbreviations: IB, irreversible bronchoconstriction; RB, reversible bronchoconstriction.
[a]χ^2 test.

Discussion

Asthma is a chronic inflammatory disease with a multifactorial background characterized by a wide range of

changes within the airway walls in association with growth and proliferation of new blood vessels. *VEGF* is a cytokine that seems to play an important role in angiogenesis in asthmatics through activation of endothelial cells, thus stimulating their migration, proliferation, and maturation and controlling the release of metabolites and inhibition of apoptosis. Ultimately, this leads to increased vascularity of the bronchial mucosa and increases the number of *VEGF*-positive cells [23-25]. Moreover, Wang et al [26] found that increased *VEGF* and *VEGF* receptor (VEGFR) expression within airway epithelial cells correlates with airway remodeling changes in histological samples from asthmatics. There are still few reports on the role of *VEGF* gene polymorphisms in the pathogenesis of asthma. One study showed that the diagnosis of asthma was positively associated with T alleles in the rs3025020 and rs3025039 single-nucleotide polymorphisms (SNPs) of the *VEGF* gene in the Chinese population [27]. There is also a report suggesting a borderline association between the G allele of the *VEGF* gene polymorphism at position –634 G/C and diagnosis of asthma in Tunisian children. Furthermore, the results suggest the possible influence of SNPs in other regions (del18 allele in the –2549 –2567 del18 variant and C allele in the +936 C/T variant) on the severity of asthma [22]. In turn, longitudinal analysis performed in the Childhood Asthma Management Program showed an association between the *VEGF* SNP rs4711750 and decreased FEV_1/FVC after around 4.5 years of observation [28]. To our knowledge, the present study is the first performed in adult asthmatics to determine a possible association between genetic variants in the promoter region of the *VEGF* gene at position –2549 –2567 del18 and irreversible bronchoconstriction.

We found that the del/del genotype prevailed in asthmatics, especially in the subgroup of patients with irreversible bronchoconstriction. In this respect, the differences between patients with irreversible bronchoconstriction and the subgroup of irreversible bronchoconstriction and the control group and between the subgroups of reversible and irreversible bronchoconstriction were nonsignificant. The highest OR for the del/del genotype was obtained for patients with irreversible bronchoconstriction compared with controls (OR=3.48); this OR was higher than that calculated for all patients with asthma (OR=2.38). It is notable that the presence of del/del was twice as likely (OR=2.05) in asthmatics with irreversible bronchoconstriction than in the subgroup of patients with reversible bronchoconstriction, despite the fact that the difference was slightly above the level of statistical significance (*P*=.163). These findings may suggest a potential role—in addition to other factors—of the *VEGF* gene polymorphism –2549 –2567 del18 in the development of asthma and the progression of airway remodeling in adult asthmatics. However, this hypothesis requires verification in a larger sample.

Predisposition to asthma is polygenic, and disease expression usually requires an interaction between genetic and environmental factors. Exposure to tobacco smoke is one of the commonest environmental factors that may exacerbate asthma symptoms and induce irreversible bronchoconstriction [11,29-31]. Our results also confirmed the association between smoking and the occurrence of irreversible bronchoconstriction. It is worth emphasizing, however, that as many as 65% of patients from that subgroup never smoked cigarettes and that the smoking index was relatively low. Disease duration also seems to play an important role, while age and gender did not seem to be significant. We confirmed the importance of this kind of complex interaction in a study where we showed that the presence of the SNP +915G/G at codon 25 in the *TGFB1* gene, when coincident with other factors, may predispose to the development of irreversible bronchoconstriction in asthmatic patients [29].

The main limitation of our study was that it was not possible to use multivariate analysis owing to the relatively small sample size and the vast predominance of del18 in the subgroup of irreversible bronchoconstriction. Therefore, the study is to be continued in order to achieve a much larger sample.

In conclusion, we present our findings to draw attention to the potential—and currently undervalued—role of variants in *VEGF* in the pathogenesis of airway remodeling in asthma.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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