

VEGF -2549 -2567 del18 polymorphism and irreversible bronchoconstriction in asthmatics

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Summary

Background: While the importance of vascular endothelial growth factor (VEGF) for the pathogenesis of several diseases (e.g. neoplasms) has been proven, the corresponding data for asthma, especially those relating to the potential associations between VEGF genetic variants and airway remodeling are relatively scarce.

Objectives: This study was purposed to evaluate the possible connection between a genetic factor which is polymorphism del/ins in the VEGF-promoter region and airway remodelling potential in asthmatics with and without irreversible bronchoconstriction.

Materials and methods: In our study participated 82 patients with asthma (therein 42 patients with irreversible bronchoconstriction) and group of 40 controls. DNA was isolated from peripheral blood leukocytes. The polymerase chain reaction method (PCR) was used for typing of the VEGF (18-bp deletion/insertion) gene polymorphism at -2549 -2567 loci. Other factors (i.e. smoking, disease duration) also were taken under consideration.

Results: The del/del genotype was found in 74.39% patients with asthma ($p=0.031$; OR=2.38), 80.95% patients with irreversible bronchoconstriction ($p=0.012$; OR=3.48) and 67.5% patients with reversible bronchoconstriction ($p=0.251$; OR=1.70). Proportion of tobacco smokers to nonsmokers was higher ($p=0.032$) and disease duration was longer ($p=0.041$) in patients with irreversible bronchoconstriction compared to those with reversible bronchoconstriction.

Conclusions: Our results showed that the presence of del18 genotype at -2549 -2567 position in the promoter of VEGF gene, along with disease duration and other factors like cigarette smoking, associate with the risk of irreversible bronchoconstriction in asthmatics.

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 diversos 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 one of the angiogenic factors produced by various types of cells. Its production remains under genetic control – the human VEGF gene has the chromosomal locus on 6p21.3 and contains a 14-kb coding region, which is divided to eight exons separated by seven introns. The VEGF gene expression may be regulated by some cytokines and growth factors (e.g. PDGF, EGF, TGF- β), interleukins (e.g. IL-1, IL-6), lipopolysaccharide (LPS) and oxygen deficiency in tissues (HIF) [1, 2, 3, 4].

VEGF mediates pleiotropic functions. First of all, it is a prime regulator of endothelial cell proliferation; it also plays an important role in physiological angiogenesis, is involved in Th2-mediated inflammatory response, influences the proliferation of epithelial cells, fibroblasts and tumor cells in carcinogenesis [5, 6]. Over-expression of VEGF resulting in inappropriate VEGF-induced angiogenesis affects the highly vascularized organs, including lungs [7]. It is proven, that VEGF expression in bronchoalveolar lavage fluid is higher in asthmatics than in healthy controls, while its over-expression in induced sputum and plasma is associated with the severity of the disease [8, 9]. Chetta et al. [10] suggest that mucosal neovascularization is characteristic for chronic asthma and may lead to the airways obstruction due to inverse interrelation between mucosal vascularization and airway diameter. Therefore, VEGF deserves special interest when investigating the pathogenesis of asthma and the possible changes in the airways wall in asthmatics.

Asthma is considered as a heterogeneous disease in which genetic and environmental factors contribute to the occurrence and severity of symptoms [11, 12, 13, 14]. It is characterized by chronic airways inflammation which, simultaneously with damage, activates repairing processes leading also to the VEGF-mediated neovascularization [15]. It has been already found that the polymorphic features of

the promoter region of the VEGF gene may influence the transcription potential of this gene. The meta-analysis performed within the GWAS [16] revealed that the level of VEGF in blood may be interpreted in the light of some polymorphic features within the VEGF gene. Notably, -2549 -2567 del18 polymorphism was found to be correlated with an increased VEGF production, which in turn may contribute to airway remodeling with irreversible bronchoconstriction of different severity [17, 18, 19].

In the available literature, there is a lack of reports on the presence of association between VEGF gene del/del variant with the risk of irreversible bronchoconstriction in patients with asthma. Therefore, the study on that was undertaken and presented in this paper.

Materials and methods

- *Population study*

The study sample included 122 individuals (80 females). Among them were 82 patients (54 females) with the diagnosis of asthma established according to The Global Initiative for Asthma recommendations (GINA) and 40 controls (26 females) without allergies or chronic pulmonary diseases. Asthma and/or COPD presence was determined on the basis of self-reported previous medical diagnoses, the GINA [11] and GOLD [20] guidelines, as well as 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 two subgroups according to the presence (42 patients; 25 females) or absence (40 patients; 29 females) of irreversible bronchoconstriction established on the basis of the bronchodilation test. After genotyping of VEGF polymorphism, the results provide genotype classifications: 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 the Wroclaw Medical University, Poland (No. KB 68/2011). The demographic data and clinical profiles are shown in Table 1.

- *Bronchodilation test*

In all examined groups, pulmonary function test and bronchodilation test were performed. Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1) values were established using Master Scope CT Spirometer (Erich Jaeger GmbH, Wurzburg, Germany) and showed as a percentage of predicted values. Each parameter was recorded three times and the best score was used in further analysis. Bronchodilation test was performed after inhalation with 5 mg of salbutamol (SteriNeb Salamol; Teva Pharmaceuticals, Warsaw, Poland) administrated with the jet nebulizer (Model 4650-U, Devilbiss, Heston, UK). The increase of post-bronchodilator values: FEV1 > 120 mL and > 12%, were taken as a criterion of reversible bronchoconstriction.

- *DNA isolation*

DNA was isolated from peripheral blood lymphocytes using a DNA isolation kit (QiAmp DNA Blood Mini kit, Syngen Biotech, Wroclaw, Poland) following the recommendation of the manufacturer.

- *Genotyping of VEGF's polymorphism*

All asthmatics and controls were genotyped by polymerase chain reaction method (PCR) similar with described by Lachheb et al. [22] for the verification of the occurrence of the VEGF's gene polymorphism, which comprises the addition or the loss of 18 base pairs (18 bp) within the promoter region in VEGF gene at a position -2549 -2567. The concentration of the isolated DNA and its purity was identified by spectrophotometer (NanoDrop ThermoScientific, Thermo Fisher Scientific, Wilmington, USA). Then the mixture to carry out PCR reaction was prepared. The total volume of the PCR was 25 µl containing 100 ng of genomic DNA, 1 x 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, Gdansk, Poland); the final MgCl₂ concentration was 4 mmol/l. The PCR comprised an initial denaturation step (95°C for 15 min.), then 35 cycles (95°C for 30 s), primer annealing (54°C for 30 s and 72°C for 30 s) and a final extension step (72°C for 10 min.). The primer used for -2549 -2567 del18 was: F: 5'-cctggagcgttttggttaa-3' and R: 5'-atataggaagcagctggaa-3' (DNA-primers VEGF, Polgen, Lodz, Poland). The received PCR products were subjected to electrophoresis in agarose gel stained with ethidium bromide. DNA in the form of strips was visible by fluorescence under UV light transilluminator. For the -2549 -2567 del 18 the fragment sizes were 234 bp when the insertion of 18 (bp) is present and 216 bp when the deletion of 18 (bp) is present.

- *Statistical analysis*

The data were analyzed using of statistical program Statistica 10 for Windows. Within-group comparison was made using χ^2 test. The results are presented with an odds ratio (OR) and the 95% confidence intervals (95% CI). Statistical significance refers to a p value < 0.05.

Results

The general characteristic of the 82 patients with asthma and 40 controls is shown in Table 1. The distribution of sex in both examined groups was similar (p=0.167) and both groups did not differ with respect to age (p=0.169). The disease duration was longer in patients with irreversible bronchoconstriction (p=0.041) than in subgroup with reversible bronchoconstriction.

Among examined asthmatics, twenty-one patients (25.6%) had a history of current smoking. In subgroup of asthmatics with reversible bronchoconstriction only six persons (15%) had a history of smoking but in the subgroup with irreversible bronchoconstriction fifteen patients (35.71%) had a positive history toward smoking. Proportion of smokers to nonsmokers was similar in asthmatics and in

the control group ($p=0.824$), but significantly higher in patients with irreversible bronchoconstriction compared to those with reversible bronchoconstriction ($p=0.032$) (Table 2). The mean smoking index in group of asthmatics was relatively low, equal to 11.76 pack-years.

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

There were statistically significant differences between asthmatics and controls in the distribution of del/del genotype ($p=0.031$; OR=2.38), as well as between the subgroup of patients with irreversible bronchoconstriction and individuals from the control group ($p=0.012$; OR=3.48). No statistically significant differences in the distribution of del/del genotype were shown between controls and asthmatics with reversible bronchoconstriction ($p=0.251$; OR=1.70) and between subgroups with reversible and irreversible bronchoconstriction ($p=0.163$; OR=2.05) (Table 4).

Discussion

Asthma is a chronic inflammatory disease of multifactorial background characterized by a wide range of changes within the walls of airways connected with growth and proliferation of new blood vessels. Vascular endothelial growth factor (VEGF) is one of these cytokines which seems to play an important role in angiogenesis in asthmatics by activation of endothelial cells, stimulating their migration, proliferation, maturation, controlling the release of metabolites and inhibition of apoptosis. Ultimately this leads to increased vascularity of the bronchial mucosa and increase the number of VEGF-positive cells [23 ,24, 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 taken from asthmatics. There are still few reports on the role of VEGF gene polymorphism in the pathogenesis of asthma. It was shown in one study that the diagnosis of asthma was positively associated with T alleles in rs3025020 and rs3025039 SNP of the VEGF gene in Chinese population [27]. There is also a report suggesting the borderline relationship for G allele of VEGF gene polymorphism at position -634 G/C with diagnosis of asthma in Tunisian children. Furthermore, the results presented in last mentioned paper suggest the possible influence of SNP in other regions (del18 allele in the -2549 -2567 del18 and C allele in the +936 C/T) on the severity of asthma [22]. In turn, longitudinal analysis performed in the Childhood Asthma Management Program (CAMP) showed an association of VEGF SNP rs4711750 with FEV₁/FVC decline over around 4.5 years of observation [28]. The present study is, to the best of our knowledge, the first performed in adult asthmatics to determine the possible association of genetic variants in the promoter region of the VEGF gene at position -2549 -2567 del18 with irreversible bronchoconstriction.

In our research, we found that del/del genotype prevailed in the group of asthma, especially in the subgroup of patients with irreversible bronchoconstriction. In this respect, the differences between the subgroup of irreversible bronchoconstriction and in the control group and between subgroups of reversible and irreversible bronchoconstriction were insignificant. The highest odds ratio for del/del genotype was obtained for patients with irreversible bronchoconstriction compared to controls (OR=3.48), higher than that calculated for all patients with asthma (OR=2.38). It is notable that the chance of the presence del/del was twice higher (OR=2.05) in asthmatics with irreversible bronchoconstriction than in the subgroup of patients with reversible bronchoconstriction, despite being slightly above the level of statistical significance ($p=0.163$). These findings may suggest the potential contribution (among other factors) of VEGF gene polymorphism -2549 -2567 del18 to the development

of asthma and the progression of airway remodeling in adult asthmatics. However, this hypothesis requires verification in a study conducted on a larger sample.

The predisposition to asthma is polygenic and the expression of this disease usually requires an interaction between genetic and environmental factors. One of the commonest environmental factors that may exacerbate asthma symptoms and induce irreversible bronchoconstriction is exposure to tobacco smoke [11, 29, 30, 31]. Our results also confirmed the association of smoking habit with the occurrence of irreversible bronchoconstriction. It is worth emphasizing, however, that as many as 65% of patients from that subgroup never smoked cigarettes, and the smoking index among them was relatively low. Among other factors studied, the important role seems to play the duration of the disease, while age and gender appeared not to be significant. The importance of this kind of complex interaction has been confirmed in another study, where we showed that the presence of SNP (+915G/G) at codon 25 in TGF- β 1 gene, when coincident with some other factors, may predispose to the development of irreversible bronchoconstriction in asthmatic patients [29].

Our study has a potentially significant drawback – the use of statistical methods of multivariate analysis was not possible to be implemented due 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 much larger sample.

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

Contribution statement

KG conceived the idea for the study and coordinated funding for the project. JL held mentoring. AL, EJ and WM contributed to the design of the research. All authors were involved in the data collection. All authors edited and approved the final version of the manuscript.

Conflict of interest

All authors declare no conflict of interest.

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