

Analysis of the SMAD4 gene in asthma

Communication

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Abstract: Considering the importance of the TGF- β signaling pathway for normal lung function and especially its roles in inflammation and tissue remodeling, key features of asthma pathology, it can be assumed that these molecules may harbor mutations in asthmatics. The aim of this study was to analyze the SMAD4 gene in patients with asthma. Analysis has encompassed exons 10, 11, 12 and 13 encoding the carboxy-terminal (MH2) domain of the SMAD4 protein, where mutations most frequently occur. The study included 50 patients (20 men and 30 women) with asthma aged between 17 and 73 years (average age 45.2 ± 15.6 years). Polymerase chain reaction (PCR) was used to amplify exons 10, 11, 12 and 13 of the SMAD4 gene and the obtained PCR products were subjected to direct DNA sequencing. No nucleotide changes were found in any of the analyzed exons in either of the subjects. Based on the results of this study, it seems that mutations in the carboxy-terminal (MH2) domain of the SMAD4 are not present in asthmatic patients. Future research should be directed at the analysis of the complete gene, including regulatory elements, in order to resolve the exact role of SMAD4 in asthma.

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Asthma is a chronic inflammatory respiratory disease characterized by airway remodeling and reversible airway obstruction which result in the constriction of the airways and subsequent shortness of breath, wheezing and cough [1]. Asthma is a multifactorial disease and several different genes and environmental factors are involved in its onset and development. There is a well established association between a large number of polymorphisms in different genes and asthma [2]. Although the TGF- β signaling pathway plays an important role in asthma, the role of polymorphisms in the genes encoding these molecules is poorly investigated. It is assumed that SMAD proteins, as mediators of the TGF- β signaling pathway, may play a role in asthma, but their exact role in lung pathology has not been established [3].

The SMAD4 gene encompasses 49.5kb of DNA on chromosome 18 and consists of 13 exons, 2 non-coding

and 11 coding [4]. The SMAD4 gene encodes for the common intracellular mediator of TGF- β superfamily pathway, which regulates a number of cellular processes such as proliferation, differentiation, apoptosis, migration and adhesion [5]. The SMAD4 protein has two highly conserved globular domains termed MH1 and MH2 (Mad homology domain) that are connected by a linker region. The amino-terminal MH1 domain of the SMAD4 protein plays a role in the interaction with the DNA molecule [3]. The linker region is enriched in proline residues and presents a signal for nuclear export. The carboxy-terminal MH2 domain is responsible for interaction with other SMAD proteins activated by phosphorylation, as well as for interaction with numerous transcription factors [6]. Previous studies have detected a large number of different types of SMAD4 gene mutations that affect different segments of the SMAD4

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gene in many malignant and non-malignant diseases [3, 6-9]. The analysis of the distribution of previously detected SMAD4 mutations indicates that mutations most frequently occur in the segments encoding the MH2 domain of the SMAD4 protein [10].

Until recently, mutations of the SMAD4 gene have been investigated only in sporadic malignant tumors of the gastrointestinal tract and several systemic disorders. Mutations in the SMAD4 gene are common in Hereditary Hemorrhagic Telangiectasia (HHT), Juvenile Polyposis Syndrome (JPS) and Myhre Syndrome. Recent reports have indicated that the phenomenon of SMAD4 haploinsufficiency leads to a decrease in TGF- β signal transduction, altering the expression of a broad subset of target genes. This phenomenon was associated with augmented colonic inflammation in human and mice [11, 12]. Based on these recent findings, it can be speculated that germline mutations in the SMAD4 gene may lead to development of different pathologies, depending on the severity of the mutation and relevance of the TGF- β signaling pathway for the specific tissue. Considering the importance of the TGF- β signaling pathway for normal lung function and especially its roles in inflammation and tissue remodeling, key features of asthma pathology, it can be assumed that these molecules may harbor mutations in asthmatics.

The aim of this study was to analyze the SMAD4 gene in patients with asthma and to determine whether the mutations in this gene may play a role in asthma pathology. The analysis encompassed exons 10, 11, 12 and 13 encoding the carboxy-terminal (MH2) domain of the SMAD4 protein, where mutations most frequently occur.

The study included 50 patients (20 men and 30 women) with asthma, diagnosed and treated at the Department of Pulmonology of the Zvezdara University Medical Center. The youngest patient was 17 years old, the oldest was 73 and the average age was 45.2 ± 15.6 years. All patients gave informed consent and the study was approved by the hospital's Ethical Committee. Peripheral blood of asthmatic patients was used for DNA analysis. Extraction of DNA from blood samples was performed by GeneJET Genomic DNA Purification kit (Fermentas). Polymerase chain reaction (PCR) was used to amplify exons 10, 11, 12 and 13 of the SMAD4 gene using the following primers: 5'-GGATGTTCTTTCCATT-TAT-3' and 5'-ACAATCAATACCTTGCTCTC-3' for exon 10, 5'-TATTAAGCATGCTATACAATCTG-3' and 5'-CTTCCACCCAGATTTCAATTC-3' for exon 11, 5'-AGGCATTGGTTTTAATGTATG-3' and 5'-CTGCTCAAAGAACTAATCAAC-3' for exon 12 and 5'-CCAAAAGTGTGCAGCTTGTTG-3' and

5'-ATTGTATTTGTAGTCCACC-3' for exon 13. The amplification was carried out in 50 μ L total volume containing genomic DNA (20-100 ng/ μ L), FireTaq (Solis BioDyne) (1 U/ μ L), 10x FireTaq buffer (Solis BioDyne), 25 mM MgCl₂, 2 mM of each dNTP and 10 pmol of each primer. The amplification was performed under the following conditions: 94°C for 5 min; 30 cycles: 94°C for 30 sec, 55°C for 45 sec, 72°C for 30 sec; 72°C for 10 min. Amplified fragments encompass complete exons and noncoding sequences at the exon/ intron junctions. The obtained PCR products were purified using GeneJET PCR Purification kit (Fermentas) and subjected to direct DNA sequencing with one of the primers used for amplification using BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems). Sequencing products were analyzed on a 3130 Genetic Analyzer (Applied Biosystems). Sequencing data were analyzed using the Sequencing Analysis Software (Applied Biosystems).

No nucleotide changes were found in any of the analyzed exons in either of subjects. Based on the results of this study, it seems that mutations in the carboxy-terminal (MH2) domain of the SMAD4 are not present in asthmatic patients. This finding should be taken with caution since the study sample is relatively small and it should be confirmed in a larger group of subjects. Being most commonly affected by mutations in various human pathologies and being responsible for the interaction of SMAD4 with other molecules, both proteins and DNA, MH2 is the most likely candidate region within SMAD4 gene to harbor mutations that could potentially underlay asthma pathology. On the other hand, these mutations often abolish the function of the SMAD4 protein, usually lead to severe clinical consequences and are mostly found in malignant diseases [13]. Although less common, mutations in the amino-terminus and linker region lead to less severe SMAD4 protein defects, considering that they alter protein conformation and activity rather than abolish its function. Therefore, their role in asthma should also be investigated since they may act as potential risk factors and/or phenotype modulators. In addition, recent findings indicate that the regulatory region of the SMAD4 gene may also harbor genetic variants associated with haploinsufficiency [14]. For this reason, future research should be directed at the analysis of the complete gene, including regulatory elements, in order to resolve the exact role of SMAD4 in asthma.

Conflict of interest statement

Authors state no conflict of interest.

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