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Neurotrophin serum concentrations and polymorphisms of neurotrophins and their receptors in children with asthma

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Summary
Background: In the recent years numerous studies have analysed the effects of neurotrophins on allergic inflammation in airway diseases reporting increased neurotrophin levels locally in the airways as well as in serum of asthmatic patients. We aimed to investigate if levels of neurotrophins in serum of asthmatic children are influenced by the genotype of functional variants within genes encoding analysed neurotrophins and their specific receptors.
Methods: In the study we included 98 children diagnosed with asthma. Genotyping of 9 polymorphisms located in neurotrophins genes and their receptors genes was done with use of TaqMan SNP genotyping assays or PCR-RFLP. The serum levels of four neurotrophins (BDNF, NGF, NTF3, NTF4) were analysed during exacerbation of asthma symptoms with use of DuoSet ELISA Development Kit (R&D).
Results: The two patients with the genetic variant A/A of *NTRK1* (rs6334) showed significantly higher NGF serum concentrations (113.4 and 218.1 pg/mL) as compared to the mean NGF serum concentrations in the total group of patients (34.8 pg/mL). We also observed a significant epistatic interactions between variants of *NGF* rs6330 and *NTRK1* rs6334 that influenced NGF serum level ($P = 0.0004$). Analysis of four neurotrophins serum levels in relation to different genotypes of analysed neurotrophins genes showed no significant differences among analysed asthmatic children.

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Conclusions: Our results suggest that, among analysed neurotrophins, NGF serum levels may be influenced by the genotype of *NTRK1* gene individually as well as in the interaction with *NGF* functional genetic variant suggesting their involvement in allergic inflammation in asthma. Serum levels of the other neurotrophins do not seem to be affected by the variants in the analysed genes.

Introduction

Neurotrophins are neurotrophic growth factors that stimulate neurodevelopment in the nervous system. They are also mediators of the interactions between immune and neuronal cells, integrating the neuroimmune crosstalk in the pathogenesis of allergic diseases including asthma. Apart from neurons, neurotrophins are potent to activate also structural (airway epithelium) and immune cells (eosinophils, lymphocytes, mast cells). It was reported that structural and inflammatory cells express neuronal receptors and release mediators which directly communicate with nerve endings in the airways and skin.[1] It has been also observed that allergic asthma is associated with changes in neuronal control in the airways.[2] This, in turn, may modulate allergen sensitization and allergic inflammation.[3]

Neurotrophins are polypeptides that support growth, differentiation, and survival of neurons in developing and adult nervous systems. The prototypical neurotrophin is nerve growth factor (NGF) and this family also includes brain-derived neurotrophic factor (BDNF), neurotrophin 3 and neurotrophin 4. Neurotrophins act *via* a family of receptor tyrosine kinases first identified as tropomyosin-related kinases (Trks) initiating cell proliferation and survival. NGF preferentially activates TrkA receptors, BDNF and NTF3 are selective for TrkB receptors, and NTF4 is selective for TrkC receptors. Neurotrophins also act *via* an additional receptor (p75NTR), which has no tyrosine kinase activity and, unlike Trk receptors, binds neurotrophins with low affinity and specificity,[4] however effectively binds pro-neurotrophins inducing cell death.[5]

In the recent years numerous studies have analysed the effects of neurotrophins on allergic inflammation in airway diseases. Elevated BDNF, NGF and NTF3 levels have been found in bronchoalveolar lavage fluid (BALF) after allergen challenge in allergic asthmatic patients.[2,6] Asthmatic patients demonstrated elevated levels of neurotrophins (NGF and BDNF) in serum and locally in the airways[7–9] and significant increase in neurotrophins levels in the airways was observed following allergen provocation.[6,10] Increased neurotrophins concentration (BDNF) also correlated with clinical parameters of allergic airway dysfunction such as airflow limitation and airway hyperresponsiveness in asthmatic patients,[11] whereas its level normalized after anti-inflammatory treatment with inhaled corticosteroids.[9] All neurotrophins analysed in this study were also found to upregulate the neurotrophin receptors on eosinophils from BALF following allergen challenge in asthmatic patients, resulting in increased viability of eosinophils *in vitro* after incubation with all four neurotrophins.[12] These results indicated that neurotrophin mediated activation of bronchial eosinophils might play a role in the regulation of eosinophilic inflammation in allergic asthma.

Based on the previous studies indicating that functional polymorphisms within neurotrophins and their receptors genes may alter gene expression and protein secretion, we hypothesized that neurotrophins protein levels are affected by functional polymorphisms within neurotrophins genes and their receptors altering circulating neurotrophin concentrations in asthma. Therefore, in the present study we aimed to investigate the correlation between functional variants of four neurotrophin genes and their specific receptors genes and the serum levels in asthmatic children during symptoms exacerbation.

Methods

Study design

Children diagnosed with asthma were included in this study. The neurotrophins levels were analysed during exacerbation of symptoms in the course of disease. Exacerbation was defined as presence of asthma symptoms (daytime symptoms, nocturnal symptoms, limitation of daily activities, the need for reliever treatment, reduced lung function). All participants as well as their parents have given written informed consent. Local ethics committee accepted the project. Study was performed in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Patients

The study was performed on Polish sample of 98 asthmatic patients of Caucasian origin in age from 6 to 18 years old. Patients were recruited from inpatients treated in the Department of Pediatric Pulmonology, Allergy and Clinical Immunology of Poznan University of Medical Sciences and from the Outpatient Clinic of Allergology in Rzeszow. Asthma diagnosis was made according to GINA recommendation, based on clinical asthma symptoms and lung function test. Spirometry was performed on Lung Test 1000 (MES) according to ERS/ATS guidelines.[13]

Asthma severity was based on GINA guidelines into mild, moderate and severe at least 6 months before inclusion in the study.[14]

Allergic predisposition was suggested by current or past symptoms of atopic dermatitis, allergic rhinoconjunctivitis (seasonal or perennial) or food allergy. Atopy was confirmed when children fulfilled the following criteria: total IgE level higher than the upper normal limits for age;