



Relationship between interleukin-13 rs20541 single nucleotide polymorphisms and therapeutic efficacy in children with asthma

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Abstract

Objective: To investigate the relationship between therapeutic efficacy in children with asthma and interleukin-13 (IL-13) rs20541 polymorphisms.

Methods: Fifty children with moderate-to-severe asthma were assigned to the GG, GA, and AA groups according to the IL-13 gene locus rs20541 polymorphism. The patients received budesonide inhalation suspension 1 mg twice daily combined with fluticasone propionate 80 µg/inhalation. The improvement of clinical symptoms (gasping, coughing, and wheezing), improvement of lung function, and adverse reactions were observed.

Results: Lung function did not significantly differ among three groups before treatment. After treatment, the time to symptom relief was significantly shorter in the GG group than that in the other two groups. The forced expiratory volume in one second and percent predicted peak expiratory flow were also significantly better in the GG group than in the other two groups.

Conclusion: Budesonide inhalation suspension combined with fluticasone propionate is an effective treatment regimen for moderate-to-severe asthma. Polymorphism of the IL-13 rs20541 locus may be correlated with therapeutic efficacy. Patients carrying the GG allele were more responsive than their counterparts with the GA or AA allele.

Keywords

Interleukin-13, gene polymorphism, bronchial asthma, therapeutic efficacy, budesonide, fluticasone, inhaled glucocorticoid, long-acting β_2 -adrenoceptor agonist

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Introduction

Bronchial asthma is the most common respiratory allergic disease in children. This genetic heterogeneous disease is caused by both environmental and genetic factors. It is a chronic airway inflammatory disease involving a variety of cells and cellular components. Interleukin-13 (IL-13) is a cytokine secreted by T helper type 2 cells that plays an important role in the pathogenesis of asthma. At present, a number of studies have illustrated that IL-13 gene polymorphism is associated with asthma susceptibility,¹⁻⁵ and therapies targeting this gene are also under development.

Glucocorticoids and beta-receptor agonists are effective and widely used therapeutic regimens in the current treatment of asthma. Studies have demonstrated that genetic variants of the IL-13 promoter rs1800925 allele can aggravate the condition of children with asthma who receive inhaled corticosteroids (ICSs).⁶

IL-13 gene polymorphism may affect therapeutic efficacy in children with asthma. Gene variants of IL-13 have been frequently associated with asthma. IL-13 maps to chromosome 5q, a region frequently linked to asthma, total serum IgE levels, airway responsiveness, and other asthma-related phenotypes.⁷⁻¹¹ Two of the best characterized single nucleotide polymorphisms (SNPs) in IL-13 include a promoter SNP (-1111, rs1800925) and a coding SNP in exon 4 (Arg130Gln, rs20541).¹²

To study the effect of IL-13 gene polymorphisms on treatment efficacy in pediatric asthma, we investigated the relationship between genetic polymorphisms at the rs20541 locus and the efficacy of glucocorticoids combined with beta-agonists in children with asthma.

Objectives and methods

Study subjects

Children hospitalized between September 2012 and December 2013 were selected for

study enrollment. All patients met the criteria for acute exacerbation of moderate-to-severe asthma.¹³ No children exhibited vital organ dysfunction such as heart, liver, and kidney disease, hemorrhagic disease, or congenital lesions. No patients received hormonal drugs within 1 month before admission. Patients were divided into three groups according to the rs20541 polymorphism: GG, GA, and AA. The clinical data collected included (1) the time to clinical symptom relief and (2) the improvement of lung function after treatment.

All procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This study was approved by the relevant Ethics Committee. Informed consent was obtained from the parents of all individual patients included in the study.

Diagnostic criteria

The diagnostic criteria for pediatric asthma were as follows: (1) parental history of asthma, physician diagnosis of atopic dermatitis, or evidence of sensitization to aero-allergens; (2) the presence of two signs among evidence of sensitization to foods, $\geq 4\%$ peripheral blood eosinophilia, and wheezing apart from colds.⁷

Treatment

All children were given routine treatment, including oxygen inhalation, expectorants, and treatment to correct fluid and electrolyte imbalances. Antibiotics were given to children as needed. Budesonide inhalation suspension (1 mg) combined with fluticasone propionate 80 μg /inhalation were administered twice daily.

Observation indicators

Pulmonary ventilation function tests were performed during treatment, including observation of the forced expiratory volume in one second (FEV1) and percentage predicted peak expiratory flow (PEF) during treatment. The following clinical symptoms of the three groups were recorded daily: disappearance of gasping, coughing, and wheezing and adverse reactions during treatment and the improvement of lung function and clinical symptoms after treatment versus before treatment.

Reagents

A genomic DNA extraction kit was obtained from Kangwei Century Company (<http://www.cwbiotech.com>, Beijing, China). dNTP and pfu DNA polymerase were purchased from Shanghai Shengong (<https://www.sangon.com>, Shanghai, China).

Extraction of DNA

After 2 mL of peripheral venous blood were anticoagulated using EDTA, DNA was extracted and stored at -20°C .

PCR amplification of the target fragment and gene sequencing

The following primers were designed according to the sequence of rs20541 (ACCESSION NG_012090) in GenBank using Primer 5.0: forward primer, 5'-GGGCTCAAGGGCTCCTAACT-3'; reverse primer, 5'-TCCCGCCTACCCAAGACA TT-3'. The PCR reaction conditions consisted of initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 40 s, with a final extension at 72°C for 7 minutes. The PCR product was detected using 2% agarose gel

electrophoresis, and it was sent to Shanghai Biotech (Shanghai, China) for gene sequencing.

Statistical analysis

All data were statistically analyzed using SPSS 21.0 statistical software (IBM Corp., Armonk, NY, USA). Numerical data (mean \pm SD) were analyzed using post-hoc analysis of variance and analysis of covariance, and percentages were analyzed using the chi-squared test. $P < 0.05$ denoted statistical significance.

Results

Patient characteristics

The patient cohort consisted of 28 boys and 22 girls aged 3 to 12 years (mean age, 5.45 ± 2.37 years). Regarding asthma severity, 46 patients had moderate asthma, and four patients had severe asthma. There were no significant differences in age and gender among the three groups.

Comparison of clinical symptom relief time among the three groups

According to the results of gene sequencing, the rs20541 locus features the GG, GA, and AA polymorphisms.

Clinical symptoms were alleviated by treatment in all three groups. The time to disappearance of gasping, coughing and wheezing was significantly shorter in the GG group than that in the GA and AA groups ($P < 0.05$, Table 1).

Comparison of lung function improvement among the three groups

There were no significant differences in lung function parameters among the three groups before treatment. FEV1 and PEF were significantly improved after treatment

Table 1. Comparison of the time to clinical symptom relief after treatment in each group (mean \pm SD).

| rs20541 genotype | Cases (n) | Gasping (days) | Cough (days) | wheezing (days) |
|------------------|-----------|-----------------|-----------------|-----------------|
| GG | 22 | 3.18 \pm 0.79 | 3.05 \pm 0.84 | 3.27 \pm 0.77 |
| GA | 17 | 4.94 \pm 1.02 | 5.59 \pm 0.94 | 5.41 \pm 1.42 |
| AA | 11 | 4.91 \pm 1.44 | 5.73 \pm 1.68 | 5.91 \pm 1.44 |
| P | | 0.000 | 0.000 | 0.000 |

in all groups (all $P < 0.05$). After treatment, FEV1 and PEF were significantly higher in the GG group than in the other groups ($P < 0.05$, Table 2).

Side effects

The adverse reaction in the three groups was pharyngeal discomfort, including two cases in the GG group and one case each in the GA and AA groups. There was no significant difference in the incidence rate of side effects among the three groups. No severe side effects were observed among three groups.

Discussion

Asthma is a genetically heterogeneous disease characterized by varying degrees of airway spasm, hyperresponsiveness, and chronic inflammation.¹⁴ ICSs and long-acting β 2-adrenoceptor agonists (LABAs) act on different aspects of the pathogenesis of asthma, and they have synergistic and complementary effects at the molecular and cellular levels. Therefore, the two drug classes are often used in combination to treat asthma. Inhalation of low-dose glucocorticoids produces a strong anti-inflammatory effect and reduces the rates of resistance to beta-agonists and glucocorticoids. Several small studies suggested that chronic LABA therapy with or without ICS treatment in Arg/Arg homozygotes was related to adverse outcomes, specifically worsening of lung function during therapy.^{15,16} However, a large clinical trial with a second replicate

population of patients with asthma who received LABAs in combination with ICSs revealed that the ADBR2 genotype exerted no effect on therapeutic responses including lung function and asthma exacerbations, strongly suggesting no adverse events are provoked in by LABA/ICS combination treatment in patients with moderate-to-severe asthma.¹⁷ With the correct use of ICSs in combination with LABAs and optimization of the existing drugs, the conditions of 90% to 95% of patients with asthma can be properly controlled.¹⁴ However, 5% to 8% of patients with refractory asthma remain untreated despite the standardized treatment of asthma. Targeted therapies for various cytokines (e.g., IL-4, IL-5, IL-13, IL-17) and bronchial thermoplasty are currently research hot-spots, but ICSs, LABAs and leukotriene receptor antagonists remain the preferred therapeutic agents for pediatric asthma.

IL-13 plays an important role in the pathogenesis of asthma, specifically by promoting the development of inflammation (signaling to eosinophils and B lymphocytes) and airway remodeling (signaling to fibroblasts, airway smooth muscle, dendrites and epithelial cells).¹⁸ At present, several studies have found that the IL-13 rs20541 polymorphism is associated with the genetic susceptibility to asthma, and the IL-13 1112 C>T gene mutation and its haplotype are associated with the response to and efficacy of leukotriene modulators in children with asthma.¹⁹ Genetic variation of the IL-13 promoter rs1800925 allele

Table 2. Improvement of lung function after treatment in each group.

| rs20541 genotype | Cases (n) | FEV1 (%) before treatment* | FEV1 (%) after treatment | P | PEF (%) before treatment** | PEF (%) after treatment | P |
|------------------|-----------|----------------------------|--------------------------|-------|----------------------------|-------------------------|-------|
| GG | 22 | 89.41 ± 1.71 | 96.55 ± 1.14 | 0.000 | 89.41 ± 1.71 | 96.73 ± 1.08 | 0.000 |
| GA | 17 | 88.24 ± 1.35 | 91.47 ± 2.03 | 0.000 | 88.24 ± 1.35 | 92.18 ± 1.81 | 0.000 |
| AA | 11 | 88.45 ± 1.57 | 91.91 ± 1.45 | 0.000 | 88.45 ± 1.57 | 91.73 ± 1.27 | 0.04 |

*: Before treatment, FEV1 (%) did not significantly differ among the three groups ($P = 0.57$).

** : Before treatment, PEF (%) did not significantly differ among the three groups ($P = 0.22$).

FEV1, forced expiratory volume in 1 s; PEF, percent predicted peak expiratory flow.

aggravated the condition of children who received ICSs for asthma in a prior study.⁶ The IL-13 gene polymorphism is associated with the sensitivity of patients with asthma to therapeutic drugs, affecting the time that patients achieve asthma control during drug treatment.

In this paper, we used budesonide inhalation suspension in combination with fluticasone propionate aerosol in the treatment of moderate-to-severe asthma, and patients' symptoms were effectively alleviated. The improvement of lung function in patients with the rs20541 GG allele was significantly better than that in patients with the GA or AA allele. ICSs have proven effective in the treatment of asthma over the past decades. Current guidelines for asthma management recommend low-dose ICSs as the first-line therapies for patients with mild persistent asthma and medium-dose ICSs or combination therapy with LABAs as the preferred therapies for moderately severe asthma.²⁰ Fluticasone propionate is a newer agent that exhibited greater potency in *in vitro* assays. A higher likelihood of pharyngitis was apparent when patients were treated with fluticasone at twice the dose of beclomethasone dipropionate and budesonide.²¹

Changes in the IL-13 gene rs20541 allele can affect the clinical efficacy of ICS and LABA treatment. Its polymorphism affects lung function in children with long-term asthma control and specific asthma

phenotypes. The impact is worthy of further study.

Conclusion

Budesonide inhalation suspension combined with fluticasone propionate can effectively treat patients with moderate-to-severe asthma and improve lung function. The genetic variation of the IL-13 rs20541 locus may be related to the efficacy of ICSs in combination with LABAs in the treatment of pediatric asthma. Patients carrying the rs20541 GG allele were more response to this combination regimen than those carrying the GA or AA allele. In the selection of treatment for children with asthma, the therapeutic efficacy and side effects of the agents should be considered comprehensively. At the same time, the best drug and dosage should be selected according to the rs20541 genotype of the patient, thereby enhancing the therapeutic effect, reducing the occurrence of side effects, and ensuring the effectiveness and safety of asthma treatment.

Consent for publication

Informed consent was obtained from the parents of all individual patients included in the study.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.


Ethical approval

All procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This study was approved by the ethics committee of Shanghai Pudong Hospital.

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