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ORIGINAL ARTICLE

Polymorphisms of TGFB1, TLE4 and MUC22 are associated with childhood asthma in Chinese population

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Abstract
Objective: To investigate whether the genetic variants of TGFB1, TLE4, MUC22 and IKZF3 are associated with the development of asthma in Chinese children.
Methods: 572 adolescent asthma patients and 590 age-matched healthy controls were included in this study. A total of four SNPs were genotyped, including rs2241715 of TGFB1, rs2378383 of TLE4, rs2523924 of MUC22, and rs907092 of IKZF3. Allele frequencies of the patients and the control group were compared by the Chi-square test. The Student *t* test was used to analyse the relationship between genotypes and clinical feature of the patients.
Results: Patients were found to have significantly different frequencies of allele A of rs2241715, allele G of rs2378383 and allele A of rs2523924 as compared with the controls (40.4% vs. 45.9%, $p = 0.01$ for rs2241715; 17.2% vs. 13.4%, $p = 0.01$ for rs2378383; 15.3% vs. 11.9%, $p = 0.02$ for rs2523924). For patients with severe asthma, those with genotype AA/AG of rs2241715 had remarkably higher FEV1% as compared with those with genotype GG ($59.1 \pm 4.3\%$ vs. $55.4 \pm 3.7\%$, $p < 0.001$). Moreover, those with genotype GG/GA of rs2378383 had remarkably lower FEV1% as compared with those with genotype AA ($54.6 \pm 2.9\%$ vs. $58.6 \pm 4.1\%$, $p < 0.001$).
Conclusions: Genes TGFB1, TLE4 and MUC22 are associated with the risk of childhood asthma in Chinese population. Our results associating TGFB1 and TLE4 with clinical features of asthma suggest potential application of these parameters in the management of asthma children.

Introduction

Asthma is a common chronic disease among children, with a high prevalence rate.[1,2] It is characterised by airway inflammation and bronchoconstriction followed by airflow

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obstruction.[3,4] To date, the mechanisms leading to asthma development have not been fully understood. Previous studies have identified certain clinical risk factors of asthma,[5–7] which however can only explain a small part of the disease. As indicated by the familial aggregation of asthma,[8–10] genetic determinants are promising targets to elucidate the inheritable causes of asthma. Twin studies support a strong genetic component to asthma with genetic risk factors heritability estimated to contribute to 48–79% of asthma risk.[11,12] From this perspective, identification of genetic variants associated with asthma could be helpful for a better understanding of its aetiopathogenesis.

Two traditional approaches have been applied to the identification of asthma susceptibility genes, including candidate gene association studies and linkage studies. Through these two methods, previous genetic association studies have revealed over 200 asthma susceptible genes in the past decade. However, few genetic loci show consistent associations across populations.[13,14] In recent years, it became feasible to investigate the genetic architecture of asthma by genome-wide association studies (GWASs). In the first GWAS of asthma, Moffatt et al.[15] found significant associations of SNPs adjacent to ORMDL3 and GSDMB with risk of childhood asthma in German and British populations. More recently, Himes et al.[16] found SNPs in PDE4D are associated with risk of asthma in whites from the United States and replicated this finding in two other white populations. Choudhry et al.[17] implicated chromosome 5q23 SNPs for association with asthma in patients from Puerto Ricans. Despite these intriguing findings, few genetic variants have been observed to confer large risks of asthma. It is apparent that numerous genetic risk factors are involved in the incidence of asthma with each of them conferring a small relative risk.

To date, most reported susceptibility loci were discovered in European or American populations. The different modes of linkage disequilibrium (LD) among Europeans and Asians highlight the importance of studying diverse populations in genetic association analyses. Replication of these novel loci in different populations may provide insights into racial disparities in asthma prevalence and severity. Recently, GWASs conducted in Latino population have revealed several susceptible genes of childhood asthma with genome-wide significance,[18–20] which greatly reinforced their role in the aetiology of the disease. Therefore, they are of great interest to be replicated in a different population. To the best of our knowledge, the association between asthma and the genetic variants of these genes remains obscure in the Chinese population. In this study, we aimed to investigate whether the genetic variants of TGFB1, TLE4, MUC22 and IKZF3 are associated with the development of asthma in Chinese children.

Methods

Subjects

Under the approval of the local institutional review board, the current case–control study included 572 adolescent asthma patients and 590 age-matched healthy controls. The asthma was diagnosed according to the criteria published by the American Thoracic Society,[21] and the severity was determined according to the classification of the Global Initiative for Asthma guidelines.[22] The following criteria were used for the inclusion of cases: (1) aged between 8 and 18 years; (2) being atopic. Atopic status was defined by one or more positive skin scratch test responses to common aeroallergens, or by specific IgE to any allergen >0.35 kU/l. Patients with other chronic inflammatory diseases were excluded from the study. The control group had no history of respiratory disease and no first-degree relative with asthma patients. Moreover, all the controls had a negative skin-prick result.

The baseline characteristics of the subjects were recorded, including gender, age, asthma severity, level of IgE and results of pulmonary function test (PFT). Asthma severity was rated as mild, moderate, or severe, according to symptoms of the patients.[22] PFT was performed with a Vitalograph 2150 spirometer (Compact, Buckingham, UK) and the baseline was determined by the best of three recordings. Values of forced expiratory volume in the first second of expiration (FEV1) were recorded, and we used the spirometric reference values for healthy Chinese children aged between 5 and 15 years to calculate the FEV1%.[23] All the subjects gave their informed consent for the collection of the blood samples. Blood sample was used for total IgE analysis and specific IgE analysis. Age-adjusted serum total IgE levels and specific IgE levels for common aeroallergens were determined as previously reported. The value of IgE was transformed with nature logarithm (Log 10).

Genotyping of target variants

Genomic DNA was extracted from the blood samples with a commercial kit (Qiagen K.K., Tokyo, Japan) following standard protocol. TaqMan Genotyping Assay was carried out for the genotyping of target variants. The assay was performed with each reaction unit containing 1.5 μl DNA, 5 μl TaqMan Universal PCR master mix and 3.5 μl sterile water. Thermal cycling conditions began at 95 °C for 10 min and then proceeded with 45 cycles of 92 °C for 15 s and 60 °C for 5 min. The four asthma-related SNPs reported in the Latino populations were selected for genotyping, including rs2241715 (A/C) of TGFB1, rs2378383 (G/A) of TLE4, rs2523924 (A/G) of MUC22, and rs907092 (A/G) of IKZF3. The results of genotyping assay were analysed by ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Twenty percent of the samples were randomly selected for a duplicate for the high quality of the genotyping assay.

Functional annotation

To explore the regulatory properties of the susceptible variant, we used chromatin state segmentation in LCL data generated by the ENCODE project to determine whether the variants or its proxies can annotate enhancer elements or putative transcription factor binding.

Statistical analysis

SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Baseline characteristics of the cases

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