Original Article

CTLA-4 gene polymorphisms and susceptibility to chronic obstructive pulmonary disease

Lijing Deng1*, Haixia Zhou2*, Jing Yang2, Jun Xiao2, Bo Wang2, Lan Wang2, Xuemei Ou2, Yulin Feng2

1Department of Intensive Care Unit, West China Hospital, Sichuan University, Chengdu, Sichuan Province, China; 2Department of Respiratory Medicine, West China Hospital, Sichuan University, Chengdu, Sichuan Province, China. * Equal contributors.

Received September 1, 2013; Accepted September 27, 2013; Epub October 15, 2013; Published November 1, 2013

Abstract: Objective: The aim of this study was to investigate whether four single nucleotide polymorphisms (SNPs) in CTLA-4 gene are associated with chronic obstructive pulmonary disease (COPD) in a Chinese population. Methods: Samples were collected from a Chinese population and analyzed for the association of SNPs in CTLA-4 gene with COPD in a case-control study. Four SNPs (rs231775, rs3087243, rs231725, rs5742909) in CTLA-4 gene were chosen and genotyped. The results were then analyzed using statistical methods. Results: We found that none of these four SNPs (rs231775, rs3087243, rs231725, rs5742909) in CTLA-4 gene were associated with the disease. Conclusion: Our data suggested that there was no significant association between these four SNPs in CTLA-4 gene and COPD susceptibility in a Chinese population.

Keywords: CTLA-4, chronic obstructive pulmonary disease, polymorphisms

Introduction

CTLA-4 was validated to locate on chromosome 2q33, which showed variants in the CTLA-4 gene that were associated with chronic bronchitis in COPD cases [1]. Some studies had identified the associations of CTLA-4 SNPs and several autoimmune diseases and multiple types of cancer, respectively [2-5]. To our knowledge, only little information is available on the association between CTLA-4 polymorphisms and genetic susceptibility to COPD. In this study, we have studied the relationship between the CTLA-4 polymorphisms and the development of COPD, smoking status, and lung function decline in a case control study derived from the general Chinese population.

Chronic obstructive pulmonary disease (COPD) is a major cause of high mortality and morbidity worldwide and many people suffer from this disease for years and die prematurely of it or its complications [6]. It is chronic and progressive, with a pathologic conditions comprising emphysema, lung tissue, and airway inflammation by cell-mediated proteolytic destruction related to repeated infection [7, 8]. Although smoking is a significant environmental cause of COPD, there is considerable variability in the susceptibility of smokers to develop COPD, and non-smokers can also get the disease even after eliminating the influence of passive smoking. These indicate that the genetic factors might contribute to the individual susceptibility. Currently, modern molecular methods have been adopted to identify the susceptibility factors for COPD.

T-cell activation, proliferation as well as differentiation, play a key role in the pathogenesis of COPD [9]. CTLA-4 is a T cell activation molecule essential for normal homeostasis of T cell reactivity. Engagement and cross-linking of CTLA-4 - B7 interaction enhances autoreactive and tumor-specific T cell activity [10, 11].

Recently, genomewide linkage analysis of the Boston Early-Onset COPD Study families demonstrated a significant linkage peak on chromosome 2q [12, 13] and CTLA-4 was validated to locate on chromosome 2q33, which showed variants in the CTLA-4 gene that were associ-
Association of CTLA-4 gene polymorphisms with COPD

Table 1. Characteristics of the study population

<table>
<thead>
<tr>
<th>Variables</th>
<th>COPD patients (n=148)</th>
<th>Controls (n=150)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>63.02±9.37</td>
<td>65.58±8.53</td>
<td>0.086</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>101/47</td>
<td>119/31</td>
<td>0.118</td>
</tr>
<tr>
<td>FEV1 observed (L)</td>
<td>1.66±0.63</td>
<td>1.03±0.25</td>
<td>0.01</td>
</tr>
<tr>
<td>FEV1/FVC (%)</td>
<td>59.83±6.81</td>
<td>48.39±10.58</td>
<td>0.01</td>
</tr>
<tr>
<td>Ex-smokers/Smoking (n)</td>
<td>46/102</td>
<td>40/110</td>
<td>0.223</td>
</tr>
</tbody>
</table>

COPD, chronic obstructive pulmonary disease; n, number of subjects; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; Data are presented as Mean±SD or %, unless otherwise indicated. *Obtained from Chi-squared test or unpaired t-test, as appropriate.

Table 2. Primers, amplicon conditions and restriction enzymes used in this study

<table>
<thead>
<tr>
<th>refSNP ID</th>
<th>SNPs</th>
<th>Primer sequence</th>
<th>Annealing (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs231775</td>
<td>+49A/G</td>
<td>ACGTTGGATGGAAGACACAGCTCAATGAAC</td>
<td>48.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ACGTTGGATGGAAGACACAGCTCAATGAAG</td>
<td></td>
</tr>
<tr>
<td>rs3087243</td>
<td>A/G</td>
<td>ACGTTGGATGGTCTCTTTCACCACACTTTG</td>
<td>49.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ACGTTGGATGGTCTCTTTCACCACACTTTG</td>
<td></td>
</tr>
<tr>
<td>rs231725</td>
<td>A/G 3’ flank</td>
<td>ACGTTGGATGGAAGACACAGCTCAATGAAC</td>
<td>47.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ACGTTGGATGGAAGACACAGCTCAATGAAC</td>
<td></td>
</tr>
<tr>
<td>rs5742909</td>
<td>-318C/T</td>
<td>ACGTTGGATGGAAGACACAGCTCAATGAAC</td>
<td>46.2</td>
</tr>
</tbody>
</table>

SNP, single nucleotide polymorphism.

Materials and methods

Ethics statement

This study was reviewed and approved by the Research Ethics Committee of the West China Hospital of Sichuan University and written informed consent was obtained from all subjects. The objective and procedures of this study were explained to all of the subjects. All of the subjects signed the informed consent forms. All potential participants who declined to participate or otherwise did not participate were eligible for treatment and were not disadvantaged in any other way by not participating in the study.

Subjects

A total of 148 COPD patients and 150 control subjects were included in this case-control association study. All cases and controls were unrelated individuals and from an epidemiological survey conducted in the southwestern area of China, in which 8500 unrelated Han people, age 40 or over, were randomly selected and questionnaire investigation, physical examination and pulmonary function test were done to all of them. Peripheral venous blood sample of 5 ml were drawn from each individual by standard venepuncture.

Inclusion criteria for COPD subjects were as follows: age ≥40, physician-diagnosed COPD, pulmonary function test showing post-bronchodilator forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) of less than 70% and FEV1 of less than 80% predicted [14]. Patients were excluded from the study if they had an established diagnosis of asthma, lung cancer, a history of atopy and known AAT deficiency. Patients with acute exacerbations four weeks preceding study assessment were also excluded. Disease severity was classified according to the criteria of Global Initiative for Chronic Obstructive Lung Disease (GOLD) [15]. Inclusion criteria for control patients were age ≥40 and normal pulmonary function, FEV1 predicted...
Association of CTLA-4 gene polymorphisms with COPD

≥80% and FEV1/FVC% ≥70%. Exclusion criteria for controls were as described for cases and also included a family history of COPD. Efforts were made to match cases by age, gender and smoking history.

SNPs selection and genotyping

Four SNPs (rs231775, rs3087243, rs231725, rs5742909) in CTLA-4 gene were chosen, which were in the region of chromosome 2q33 and were reported to be partly significantly associated with COPD [16].

Genomic DNA was extracted from blood using the commercially extraction kit (Tiangen Biotech Co., LTD, Beijing, China) according to the manufacturer’s instructions. PCR primers, conditions and restriction enzymes were shown in Table 2. Genotyping was carried out commercially by BGI (Shenzhen, China) using Sequenom’s iPLEX SNP genotyping protocol developed for measurement with the MassARRAY mass spectrometer [17]. Genotyping was blind to case or control status of samples. As a quality control measure, approximately 5% of samples were genotyped in duplicate to check for concordance. In addition, a selection of samples was also genotyped using restriction enzyme digestion or direct sequencing to confirm the genotyping results from BGI.

Statistical analysis

Genotype and allele frequencies of all polymorphisms were compared between cases and controls using the $\chi^2$ test, and odds ratio (OR) and 95% confidence intervals (CI) were reported to evaluate the effects of any difference between allelic and genotype distribution. Demographic and clinical data between groups were compared by the $\chi^2$ test or the Student’s t test. Hardy-Weinberg equilibrium was evaluated by the $\chi^2$ test. The SPSS package (version 13.0; SPSS Inc, Chicago, IL) was used for statistical analyses, and a two-sided $p$ value <0.05 was taken as the level for statistical significance.

Results

Demographic characteristics and results of quality control

We firstly summarized the demographic data and baseline characteristics of the study gro-
The genotypes and allele frequencies of these four SNPs in the studied groups are presented in Table 3. The allele and genotype frequencies of all the four SNPs in CTLA-4 gene did not differ significantly between the COPD patients and the controls.

Discussion

COPD involves chronic inflammation of the lower airways and may be an immune-mediated condition [18, 19]. T-cell activation and proliferation were found to be involved in pathogenesis [9]. Previous study has reported that CTLA-4 -318C/T (rs5742909) and CD86 -1057G/A polymorphisms were associated with COPD in a Chinese population [16]. However, in our study, we demonstrated that the allele and genotype frequencies of all the four SNPs in CTLA-4 gene did not differ significantly between the COPD patients and the controls [20].

Traditionally, the A allele in CTLA-4 had been identified as protective, whereas the G allele
was associated with greater susceptibility to autoimmune diseases [21]. The polymorphism (rs231775) was linked to reduced expression of CTLA-4 on the T-cell surface and impairs inhibitory function and contributes to the pathogenesis of some autoimmune diseases and multiple types of cancer [4, 22-24]. However the results were often controversial as previously reported by different research groups [2, 25, 26]. For example, association of CTLA-4 +49A/G single nucleotide polymorphisms (SNP) with COPD between the International COPD Genetics Network Family and Bergen population was reported [1]. In another study, no significant associations were found between +49A/G SNP in COPD patients and controls [16]. Our results demonstrated that there was also no association of CTLA-4 +49A/G with COPD. We were unable to fully explain this discrepancy [16]: it might be genetic heterogeneity, ethnic and geographic variations that could change frequency of specific polymorphisms in different regions, and dissimilar genetic or etiologic contribution to different diseases. Additional studies are needed to clarify this issue.

The design of this study, a case-control study nested in a relative large cohort, minimizes many possible biases and issues of comparability of the case and control groups that are of concern in other study designs. It also allows us to expand the study as cases accrue so that we may address several issues that are still of concern. We plan an expanded study that will include more people and will have a sufficient sample size to conduct more genotyping assay, which is critical to reveal the internal association between SNP in virulence gene and its related disease.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China grants 81070003, and 31071009.

Disclosure of conflict of interest

The authors have no financial conflicts of interest.

Address correspondence to: Dr. Yulin Feng, Department of Respiratory Medicine, West China Hospital, Sichuan University, 37# Guo-Xue-Xiang, Chengdu 610041, Sichuan Province, China. Tel: +86-28-85423660; Fax: +86-28-85423660; E-mail: yulin-feng2@sina.cn; Lijing Deng, Department of Intensive Care Unit, West China Hospital, Sichuan University, Chengdu, Sichuan Province, China. E-mail: deng_lijing@163.com

References

Association of CTLA-4 gene polymorphisms with COPD


