Genetic analysis of IREB2, FAM13A and XRCC5 variants in Chinese Han patients with chronic obstructive pulmonary disease

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\textbf{A B S T R A C T}

Recently, variants (rs2568494, rs2869967 and rs3821104) in the IREB2, FAM13A and XRCC5 genes were found to be associated with chronic obstructive pulmonary disease (COPD) in non-Asian populations by genome-wide association study (GWAS) analysis. To evaluate whether variants in these genes are related to COPD in Chinese Han population, we investigated COPD patients of Chinese Han ethnicity from Mainland China. Significant differences in genotypic distributions ($\chi^2 = 6.319$, $p = 0.042$ for rs2869967; $\chi^2 = 6.062$, $p = 0.048$ for rs3821104) and allele distributions ($\chi^2 = 4.014$, $p = 0.045$ for rs2869967; $\chi^2 = 5.607$, $p = 0.018$ for rs3821104) were observed between patients and control subjects for variants rs2869967 and rs3821104, whereas no statistically significant associations for genotypic and allelic distribution between IREB2 rs2568494 and COPD phenotype ($p > 0.05$) were identified. Our results support that FAM13A rs2869967 and XRCC5 rs3821104 are associated with COPD in Chinese Han population.

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1. Introduction

Chronic obstructive pulmonary disease (COPD, MIM 606963) is a genetically complex human disease that is characterized by a reduction in lung function with airflow obstruction that is not fully reversible [1]. This disorder is expected to be the third leading cause of worldwide mortality and the fifth leading cause of morbidity by the year 2020 [2]. Cigarette smoking is clearly the major environmental risk factor for the development of COPD [3]. It is well recognized that COPD has a genetic component as well as environmental, and that is may account for these differences in susceptibility. Recently, genome-wide association study (GWAS) revealed a statistically significant association between COPD and several gene variants, including the iron-responsive element-binding protein 2 gene (IREB2, MIM 147582) rs2568494, the family with sequence similarity 13 member A gene (FAM13A, MIM 613299) rs2869967 and the X-ray repair cross-complementing protein 5 gene (XRCC5, MIM 194364) rs3821104 in non-Asian populations [4–9]. To determine the association between these variants and COPD in Chinese Han population, we screened DNA of Chinese COPD patients and normal controls from Mainland China.

2. Materials and methods

2.1. Study subject

Two hundred and seventy-five unrelated Chinese Han COPD patients (age: 60.9 ± 9.7 years; male/female: 192/83) and 434 normal controls (age: 61.2 ± 9.9 years; male/female: 307/127) from Mainland China were included in the study. COPD was diagnosed according to the criteria established by the NHLBI/WHO Global Initiative for COPD (GOLD) [1]. The control group consisted of 434 unrelated subjects with healthy pulmonary function and no known medical illnesses or family disorders. Control subjects were individuals for a health check-up or community volunteers. They were matched for age, gender, ethnicity and smoking history with the case group. The characteristics of the study population were shown in Table 1. The protocol of this study was approved by the Ethics Committee of the Third Xiangya Hospital, Central South University, and each participating individual has signed an informed consent.

2.2. Genetic analysis

Genomic DNA was extracted from peripheral blood using standard phenol–chloroform method [10]. PCR amplified the IREB2
rs2568494, FAM13A rs2869967 and XRCC5 rs3821104 fragments by using a 9700 Thermal cycler System (ABI) with primers (Table 2), for 35 cycles at 95 °C for 35 s, 58 °C for 30 s, 72 °C for 35 s, and a final extension step at 72 °C for 5 min. 100 ng of gDNA, 10 pmol primers and 1 U Taq polymerase were used in a 25 μl reaction volume. 8.5 μl PCR products were digested by 0.8 U shrimp alkaline phosphatase (SAP) and 8 U exonuclease I (Fermentas) in a 10 μl reaction volume, and sequenced with an 8-capsillary 3500 genetic analyzer (Applied Biosystems, Inc.) [11]. The Hardy–Weinberg equilibrium was performed as to ascertain the normal heterogeneity of the population [12]. Pearson’s χ² tests were applied to test for significance in differences of gene frequencies. Two-tailed p value less than 0.05 was considered statistically significant [13].

3. Results

Loci information and allele frequencies were presented in Table 3. The genotype frequencies of all tested single nucleotide polymorphisms (SNPs) were in accordance with the Hardy–Weinberg equilibrium in controls. Statistically significant association with COPD compared to normal controls was observed in genotypic distributions (χ² = 6.319, p = 0.042 for rs2869967; χ² = 6.062, p = 0.048 for rs3821104) and allele distributions (χ² = 4.014, p = 0.045 for rs2869967; χ² = 5.607, p = 0.018 for rs3821104) for the FAM13A rs2869967 and XRCC5 rs3821104, consistent with the prior reports [6,8]. However, there was no significant association between the IREB2 rs2568494 variant and COPD phenotype (χ² = 0.590, p = 0.744 for genotypic distribution; χ² = 0.034, p = 0.854 for allele distribution) (Table 3), although the association was observed in White and African American patients with COPD [7].

4. Discussion

Though smoking is a well-recognized risk factor for COPD, only 15% of smokers develop the disease [14]. In addition, a minority of lifetime nonsmokers develop COPD. Accordingly, an inherent susceptibility to COPD is persuasive [15]. The genetic architecture of COPD is likely to be complex, with the contribution of environmental factors and multiple genes [14]. A variety of approaches, including candidate-gene association studies, linkage analysis, and rare-variant studies, have been used to search for COPD susceptibility loci, but with the exception of a relatively rare monogenic disorder that has been identified to be associated with COPD (alpha-1 antitrypsin deficiency), few have been consistently replicated [16,17].

Advances in Human Genomic Project and HapMap offer an opportunity to study the genetic architecture of COPD from the genomic level [18]. Recently, variants (rs2568494, rs2869967 and rs3821104) in the IREB2, FAM13A and XRCC5 genes were found to be associated with COPD in non-Asian populations by GWAS analysis.

Given the reported association between the three variants and COPD, we investigated these variants in our well-characterized cohort of 275 Chinese Han patients with sporadic COPD and 434 sex, age and ethnicity matched normal controls. Statistically significant association with COPD compared to normal controls was observed in genotypic distributions (χ² = 6.319, p = 0.042 for rs2869967; χ² = 6.062, p = 0.048 for rs3821104) and allele distributions (χ² = 4.014, p = 0.045 for rs2869967; χ² = 5.607, p = 0.018 for rs3821104) for the FAM13A rs2869967 and XRCC5 rs3821104, although no significant association was found for IREB2 rs2568494 variant.

The FAM13A gene, mapped to chromosome 4q22.1, contains 25 exons spanning about 332 kb. There have 2 splice variants of FAM13A. Variant-1 (V1) encodes a 683-amino acid protein with 2 coiled-coil domains and 3 nuclear localization signals. V2 encodes a deduced 1023-amino acid protein that has an N-terminal extension containing a RhoGAP domain that is absent in V1 [19]. The 5-kb V1 transcript ubiquitous expressed with highest expression in lung, skeletal muscle, thymus and brain. The 6-kb V2 transcript was less abundant and was detected predominantly in lung, thymus, kidney, pancreas and liver. FAM13A (also known as FAM13A1) has a putative role in signal transduction, and SNPs especially those lie in an intrinsic region downstream of a Rho GTPase–activating protein (RhoGAP) domain may be involved in COPD susceptibility [6,19]. Gene expression analyses in cell lines from several tissues (not including the lung) have revealed a consistent increase of expression in response to hypoxia although little is known about FAM13A function [20].

The XRCC5 gene, mapped to chromosome 2q35, contains 21 exons spanning about 97 kb. It encodes a deduced 732-amino acid protein, an 80-kD subunit of the Ku autoantigen, a heterodimer protein which is also known as ATP-dependant DNA helicase II or DNA repair protein XRCC5, contributing to genomic integrity through its ability to bind DNA double-strand breaks and facilitate repair by the nonhomologous end joining (NHEJ) pathway. Ku is the DNA-binding component of the DNA-dependent protein kinase, and it functions together with the DNA ligase IV-XRCC4 complex in the repair of DNA double-strand break by non-homologous end joining and the completion of V(D)J recombination events. There are several potential mechanisms for the role of XRCC5 in the development of COPD, specifically early-onset COPD. These evidences include that Ku86−/− mice also developed pulmonary emphysema early aging and an autoimmune component Ku80/86 has been to proposed to COPD, similar roles in systemic lupus erythematosus and other autoimmune disease [8].

The frequencies of the rs2869967 C-allele and rs3821104 T-allele were found to be significantly increased. Intriguingly, the association between FAM13A rs2869967 C-allele and COPD susceptibility is supported by a recent findings that variant rs7671167 in this gene is also related to lung function [4,21]. These two variants do not represent monogenic causes of COPD, and they do appear to confer increased risk for the disease. They may act as functional
mutations or variants in linkage disequilibrium with a nearby functional locus. The mechanism may be involved in the regulation of gene expression or affect transcription factor binding, native splicing, or other mechanisms that result in a decreased lung function due to reduced gene expression or affect transcription factor binding, native splicing, or other mechanisms that result in a decreased lung function.

To our knowledge, this is the first study which assesses the role of the rs2568494, rs2869967 and rs3821104 variants in a large cohort of Chinese Han patients with sporadic COPD. Larger-scale studies in different populations are warranted to document the conclusive evidence of the effects of the FAM13A rs2869967 and XRCC5 rs3821104 on COPD risk. In addition, functional studies will be required to determine the potential impact of FAM13A and XRCC5 variants on COPD pathophysiology. The discovery of genetic risk variants, such as those in FAM13A and XRCC5, may contribute to the eventual development of new therapeutic approaches to COPD.

Acknowledgments

This study was supported by the Fund of Hunan Province for Distinguished Young Scholar (09JJ1005), Cultivating and Supporting Plan for Excellent Doctoral Dissertation of Central South University, China (Y. Guo), Sheng Hua Scholars Program and Outstanding Youth Foundation of Central South University, China (H.D.), National Natural Science Foundation of China (30871351, 30870916), Program for New Century Excellent Talents in University of Ministry of Education of P.R. China (NCET-080563), and the Project Sponsored by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry of P.R. China (H.D.). The authors thank the participating patients and the investigators at the Third Xiangya Hospital, Central South University, for their cooperation and their efforts in collecting the genetic information and DNA specimens.

References


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Table 3

<table>
<thead>
<tr>
<th>Locus</th>
<th>Genotype or allele</th>
<th>Cases</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2568494</td>
<td>GG</td>
<td>0.782 (215)</td>
<td>0.770 (334)</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>0.196 (54)</td>
<td>0.214 (93)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>0.022 (6)</td>
<td>0.016 (7)</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>0.880 (484)</td>
<td>0.877 (761)</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>0.120 (66)</td>
<td>0.123 (107)</td>
</tr>
<tr>
<td>rs2869967</td>
<td>CC</td>
<td>0.280 (77)</td>
<td>0.251 (109)</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>0.549 (151)</td>
<td>0.498 (216)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>0.171 (47)</td>
<td>0.251 (109)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.555 (305)</td>
<td>0.500 (434)</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>0.445 (245)</td>
<td>0.500 (434)</td>
</tr>
<tr>
<td>rs3821104</td>
<td>TT</td>
<td>0.898 (247)</td>
<td>0.839 (364)</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>0.102 (28)</td>
<td>0.154 (67)</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>0.000 (0)</td>
<td>0.007 (3)</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>0.949 (522)</td>
<td>0.916 (795)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.051 (28)</td>
<td>0.084 (73)</td>
</tr>
</tbody>
</table>

The number in parentheses of genotype rows are the number of individuals; the number in parentheses of allele rows are the number of chromosomes; p values show the comparison results between patients and normal controls; Significant (p < 0.05) results are shown in bold.


