The effect of *CYP3A4* genetic variants on the susceptibility to chronic obstructive pulmonary disease in the Hainan Han population

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Abstract

Purpose

Genetic polymorphisms act crucial role in chronic obstructive pulmonary disease (COPD) progression. This study was designed to investigate the correlation between *CYP3A4* variants and COPD risk.

Methods

We carried out a case-control study of 821 individuals (313 patients and 508 healthy subjects) to identify the correlation of *CYP3A4* SNPs with COPD risk in the Hainan Han population. The association was assessed by Odds ratios (OR) and 95% confidence intervals (CI).

Results

Our study showed that rs4646437 polymorphism was related to an increased susceptibility to COPD (OR = 1.45, 95% CI = 1.10-1.90, $p = 0.008$). Stratified analyses indicated that the rs4646437 polymorphism was significantly associated with an increased risk of COPD in males (OR = 1.95, 95% CI = 1.19-3.20, $p = 0.008$). However, rs4646440 played a protective role in females (OR = 0.54, 95% CI = 0.31-0.93, $p = 0.024$). The rs4646437 SNP was identified significantly improve the risk of COPD in smoking subjects (OR = 1.67, 95% CI = 1.12-2.48, $p = 0.011$). While rs4646440 had a significantly lower susceptibility to COPD with non-smoking individuals (OR = 0.64, 95% CI = 0.45-0.90, $p = 0.010$). Haplotype analysis revealed that $A_{rs4646440}T_{rs35564277}$ and $G_{rs4646440}T_{rs35564277}$ haplotypes of *CYP3A4* were found to reduce the risk of COPD in non-smoking (OR = 0.61, 95% CI = 0.49-0.94, $p = 0.024$).

Conclusion

Our results would give some new understanding of the association between *CYP3A4* gene and COPD in the Hainan Han population.

Introduction

Chronic obstructive pulmonary disease (COPD) is a frequent pulmonary disease characterized by incomplete reversible airflow limitation usually induced by significant exposure to harmful particles or gas [1]. COPD, with high prevalence and mortality, will become the third leading cause of death in the world by 2020 according to an epidemiological survey, resulting in a huge economic and social burden, which needs to be given attention to by the whole society[2]. Although previous reports have been confirmed that cigarette smoking is the major risk exposure for COPD, host factors including genetic variants, aging and abnormal lung development also make individuals susceptible to COPD[3]. A large number of studies have been shown that gene variants such as *CHRNA3*, *CYP1A1/2*, *EGLN2*, *RAGE*, *CYP2A6/7*, *CYP2B6* and other genes were significantly associated with risk of COPD[4].

Cytochrome P450 3A4 (*CYP3A4*), together with *CYP3A5*, *CYP3A7* and *CYP3A43* are cytochrome P450 3A subfamily members and located on chromosome 7q22 [5]. Among them, *CYP3A4* is the most abundant P450 isoenzyme in human liver, involves in about 50% of the oxidative pre-systemic or systemic metabolism of drugs and makes a wide range of substrate specificity[6]. In recent years, it was observed that *CYP3A4* gene have higher expression in healthy and malignant lung tissue [7, 8]. Genome-wide association studies (GWAS) and some previous studies showed that genetic polymorphisms of CYPs were significantly associated with COPD susceptibility[9-13]. However, there is not any report about the relationship between *CYP3A4* variants and COPD. *CYP3A4*, as the member of CYPs, has been demonstrated that genetic polymorphisms of *CYP3A4* could significantly influence the *CYP3A4* activity[14]. And *CYP3A4* variants have been identified to be associated with risk of diseases, such as HIV infection, breast cancer, type 2 diabetes mellitus, prostate cancer, lung function and so on[15-20].

Thus, four SNPs (rs3735451, rs4646440, rs3556427, and rs4646437) in the *CYP3A4* gene were selected and checked by databases of 1000 genomes project and the MassARRAY platform in this study. Then we tried to assess the correlation between *CYP3A4* SNPs and COPD risk in the Hainan Han population. In addition, we also investigated the *CYP3A4* variants on the risk of COPD in stratified subgroups including gender and smoking status with the stratification analysis. Our present work will help to improve awareness of the molecular mechanisms of COPD and provide a fresh perspective for prevention and diagnosis of COPD.

Methods & Materials

Study subjects

In our present study, we recruited 313 unrelated Chinese COPD patients from the Hainan General Hospital. COPD was newly diagnosed in terms of the criteria established by the Global initiative for Chronic Obstructive Lung Disease (GOLD) / COPD[3]. 508 control individuals were randomly selected from the age-matched healthy population who had a routine physical examination in the same hospital. All participates must follow the exclusion criteria: 1) History of other diseases such as bronchiectasis, tuberculosis, lung tutor, pulmonary fibrosis, heart disease and other respiratory diseases. 2) Unable to perform a chest X-ray examination and pulmonary function tests for any reason. In addition, the controls have normal pulmonary function (volume in 1 second/forced volume capacity > 0.7) and no general medical diseases or genetic disorders.

Each volunteer has notified the purpose of sample collection and written informed consent was obtained from them before this study. The participants' basic characteristics such as age, gender, smoking status, body mass index (BMI) and forced expiratory volume in 1 second (FEV1) / forced volume capacity (FVC) were recorded using standardized questionnaires, are shown in Table1. All experiments were carried out depending on the protocol of Helsinki's Declaration and were approved by the Ethics Committee of the Hainan General Hospital.

SNPs selection and genotyping

Four polymorphisms (rs3735451, rs4646440, rs3556427, and rs4646437) in *CYP3A4* were selected from the database of 1000 Genomes Project. Minor allele frequency (MAF) among all SNPs was > 0.05 in the HapMap of Chinese Han Population. Genomic DNA of whole-blood samples was extracted with whole-blood genomic DNA extraction kit (GoldMag, Xi'an, China). The purity and concentration of genomic DNA were tested using NanoDrop 2000C spectrophotometer (Thermo Scientific, Waltham, USA). PCR primers in this study were designed by Agena Bioscience Assay Design Suite software (Version 2.0), and showed in Table 2. Agena MassARRAY iPLEX platform was applied for identifying SNP genotyping. The data was managed and analyzed by Agena Bioscience TYPER version 4.0 software.

Statistical analysis

We used the SPSS version 17.0 software to perform statistical analysis. All statistical tests were two-tailed and $p < 0.05$ indicated statistical significance. The differences in demographic and clinical characteristics between the case and control participants were analyzed by t-test and χ^2 tests. Hardy-Weinberg equilibrium (HWE) of SNPs in controls was evaluated with the exact test. The allele and genotype distribution in cases and controls was compared with a χ^2 test or exact test. ORs and 95% CIs were used to evaluate associations between *CYP3A4* variants and COPD susceptibility using logistic regression analysis with or without adjustment for age and gender. Multiple genetic models (co-dominant, dominant, recessive and log-additive) were tested by PLINK software. Stratified models were applied for subgroup analysis in male and female, smoking and non-smoking participants. Linkage disequilibrium (LD), haplotype construction and genetic associations of polymorphism loci were estimated by Haploview software (Version 4.2) and logistic regression analyses.

Results

General characteristics of the study participants

The basic characteristics of participants in our present study were listed in Table1. Total of 821 subjects were recruited including 313 patients with COPD (238 males and 75 females) and 508 healthy controls (337 males and 171 females). The average age was 71.80 ± 10.09 years in cases and 60.05 ± 6.48 years in controls. There were no significant differences on the smoking status and BMI distribution among case and control group ($p = 0.082$ and $p = 0.587$, respectively).

Association between *CYP3A4* variants and COPD risk

Detail information and allele frequencies of selecting SNPs are described in Table3. Four SNPs (rs3735451, rs4646440, rs35564277 and rs4646437) of the *CYP3A4* gene were successfully genotyped in this study. The observed genotype frequency of rs3735451 was not in agreement with HWE in control populations ($p < 0.05$). Multiple genetic models adjusted for age and gender were analyzed for examining the relationship between SNPs and COPD risk. Our results revealed that SNP rs4646437 exhibited more significant difference than other SNPs in allele or genotype frequency between case and control individuals (Table 3, Table 4). We found that rs4646437 polymorphism was associated with a significant

increased susceptibility to COPD under allele model (A vs G, OR = 1.45, 95% CI = 1.10-1.90, *p* = 0.008), co-dominant model (AG vs GG, OR = 1.53, 95% CI = 1.02-2.28, *p* = 0.039) and dominant model (AG-AA vs GG, OR = 1.49, 95% CI = 1.00-2.20, *p* = 0.048). Another two SNPs (rs4646440 and rs35564277) were not observed a significant association with the risk of COPD.

Stratification analysis

We evaluated the association between *CYP3A4* variants and COPD risk with stratified analysis according to gender and smoking status. Stratified analysis by gender indicated that the rs4646437 polymorphism was significantly related to an increased risk of COPD in males (A vs G, OR = 1.62, 95% CI = 1.19-2.22, *p* = 0.002; AG vs GG, OR = 1.95, 95% CI = 1.19-3.20, *p* = 0.008 and AG-AA vs GG, OR = 1.89, 95% CI = 1.17-3.05, *p* = 0.010, Table 5). However, rs4646440 was a protective role in females (A vs G, OR = 0.54, 95% CI = 0.31-0.93, *p* = 0.024, Table 5). We also examined the relationship of *CYP3A4* SNPs and COPD risk under smoking status subgroup. As is summarized in Table 6, the rs4646437 SNP was found to significantly improve the risk of COPD in smoking subjects (A vs G, OR = 1.67, 95% CI = 1.12-2.48, p = 0.011; AG vs GG, OR = 2.03, 95% CI = 1.02-4.04, *p* = 0.043 and AG-AA vs GG, OR = 2.03, 95% CI = 1.03-3.99, *p* = 0.042). While rs4646440 had a significantly lower susceptibility to COPD with non-smoking individuals (A vs G, OR = 0.64, 95% CI = 0.45-0.90, p = 0.010; AG vs GG, OR = 0.61, 95% CI = 0.38-0.98, *p* = 0.039 and AG-AA vs GG, OR = 0.59, 95% CI = 0.37-0.94, *p* = 0.027). No significant associations observed in other SNPs.

Haplotype analyses of SNPs and COPD risk

We further studied LD and haplotype analyses for exploring the association of *CYP3A4* gene with COPD risk in all subjects. LD analysis disclosed that two SNPs including rs4646440 and rs35564277 formed a LD block in non-smoking subgroup (Figure 1). Haplotype distribution between case and control group was displayed in Table 7. And the haplotype analysis indicated that $A_{rs4646440}T_{rs35564277}$ and $G_{rs4646440}T_{rs35564277}$ haplotypes of *CYP3A4* were found to reduce the risk of COPD (OR = 0.61, 95% CI = 0.37-0.99, *p* = 0.048; OR = 0.61, 95% CI = 0.49-0.94, *p* = 0.024; respectively).

Discussion

In this case-control study, we investigated the association between *CYP3A4* variants (rs3735451, rs4646440, rs3556427 and rs4646437) and COPD susceptibility in a Hainan Han population. We found that rs4646437 polymorphism associates with a significantly increased susceptibility to COPD. Furthermore, rs4646437 polymorphism significantly increased risk of COPD in male and smoking individuals. While rs4646440 showed a significant correlation with reduced susceptibility to COPD in female and non-smoking subjects. These results demonstrate that *CYP3A4* polymorphisms may play a critical role in COPD risk of Hainan Han populations. As far as we know, this is the first study to test the genetic polymorphisms of *CYP3A4* effecting on COPD risk in the Hainan Han population which may give some available information for the prevention and diagnosis of COPD.

CYP3A4 was particularly considered to be an enzyme that is the basis for certain drug induction or cascade regulation of various cytokines and their downstream signals which have a high expression level in human hepatocytes[21, 22]. In recent years, increased evidence showed that genetic variability of *CYP3A4* contributes significantly to lung function in humans[23]. That happens because the *CYP3A4* can modulate lung inflammation, the composition of airway lining fluid and the tone of pulmonary vasculature according to catalyze the oxidative metabolism and metabolic activation of most toxicants in human lung[24]. The occurrence of COPD usually caused by significant exposure to noxious particles or gases owing to pulmonary dysfunction. These suggest that *CYP3A4* variants may play an important role in the progression of COPD.

Genetic polymorphisms of the *CYP3A4* gene can influence the protein expression level, which might associate with the occurrence of diseases. Thus, we propose that the *CYP3A4* variants can affect the function of a gene resulting in the COPD progression. However, there is not any finding of correlation between *CYP3A4* variants and COPD risk. In this study, we found that rs4646437 polymorphism could dramatically increase the susceptibility to COPD in a Hainan Han population. We further examined the correlation between *CYP3A4* variants and COPD risk via stratified analysis including gender and smoking status. Upon gender-based stratification, our results showed that rs4646437 polymorphism was significantly related to an increased risk of COPD in males, while rs4646440 played a protective role in females. Stratified by smoking status, it showed that rs4646437 significantly improved COPD susceptibility in smoking subjects, while rs4646440 had a significantly lower susceptibility to COPD with non-smoking individuals. These results indicated that genetic variants of *CYP3A4* were associated with COPD susceptibility, and the risk association of the genetic variants may rely on the gender and smoking status. Cigarette smoking is the most commonly risk factor for COPD[25]. Tobacco smoke makes up of polycyclic aromatic hydrocarbons (PAHs)[26]. The activated PAHs catalyzed by *CYP3A* will reduce lung function in smokers[27, 28]. Rs4646437 and rs4646440 SNPs are located in the intron region, which might affect regulation of the *CYP3A4* gene expression in COPD with smokers. Several studies supported the genetic variants confer susceptibility by affecting *CYP3A4* gene expression[29, 30]. Thus, we conjectured that *CYP3A4* variants, especially rs4646437 and rs4646440 may affect the *CYP3A4* gene expression in the progression of COPD and need to be further explored.

Several limitations of our study are summarized as following. First, we investigated the genetic factors association with COPD susceptibility but there were not detecting gene-environment interactions in present study. Future work is needed to detect gene-environment interactions in risk of COPD. Second, we just determined role of *CYP3A4* variants in risk of COPD, further research should focus on the molecular mechanism of *CYP3A4* variants on COPD occurrence. Despite the above limitations, our present work provides the available evidence of the *CYP3A4* gene with COPD for the future study.

Conclusions

In summary, our present work indicated that the *CYP3A4* variants were associated with COPD susceptibility. Rs4646437 polymorphism were significantly associated with increasing COPD risk.

Stratified analysis showed that rs4646437 was significantly associated with an increased risk of COPD in males, while rs4646440 played a protective role in female. Rs4646437 significantly improved COPD susceptibility in smoking subjects, while rs4646440 had a significantly lower susceptibility to COPD with non-smoking individuals. In addition, the haplotype analysis indicated that $A_{rs4646440}T_{rs35564277}$ and $G_{rs4646440}T_{rs35564277}$ haplotypes of *CYP3A4* were found to reduce the COPD risk in non-smoking. Our present study will provide a useful information for prevention and diagnosis of COPD in the future.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Hainan General Hospital and with the 1964 Helsinki declaration. Informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

All authors declare that they have no competing interests.

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Authors' contributions

HF, QN, YP: Conceived and designed the experiments. JG, Q, JX, X, XZ, HN: Recruited and collected study samples. HF, XL, H: Selected the SNPs and designed primers. HF, QN: Performed the data and wrote the manuscript. HF JG, QN, YP: Contributed to data analysis and manuscript revised. All authors read and approved the final manuscript.

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