Role of matrix metalloproteinase (Stromelysin) gene in chronic obstructive pulmonary disease

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ABSTRACT

Aim: Many candidate genes for Chronic Obstructive Pulmonary Disease have been identified including the genes coding for Matrix metalloproteinases, like MMP9 and MMP12 which play an essential role in tissue remodelling and repair associated with COPD. The aim of the present study was to evaluate the association of 5A/6A promoter polymorphism of MMP3 gene with COPD from the South Indian population of Andhra Pradesh. Method: Two hundred and fifty COPD patients and 250 controls were included in the study. The MMP3 gene 5A/6A polymorphism was determined by Amplification Refractory mutation system (ARMS-PCR). Results: A significant difference was observed in genotypic frequency between patients and controls. The frequency of 5A/6A genotype was found to be significantly high in COPD in comparison with controls. Conclusion: The MMP-3 promoter 5A/6A genotype is a risk factor for COPD Patients showing its involvement in the pathology of disease.

INTRODUCTION

COPD refers to a group of diseases that block the airflow in the lungs. The exchange of oxygen and carbon dioxide becomes difficult during free breathing\[1\]. COPD is considered to be an emerging public health crisis. According to WHO COPD is the sixth most common cause of death worldwide and by 2020 it will be the third most threatening disease\[2\].

Genetic components with case-control association studies have suggested that genetic factors are important in COPD risk. A number of genes like α-Antitrypsin, Matrix Metalloproteinases (MMP-9&MP-12), Tissue inhibitory Metalloproteinase-2 (TIMP-2), Hemoxygenase-1 (HMOX-1), Microsomal Epoxide (EPHX1), Tumor necrosis factor-α (TNF-α) and surfactant protein B (SFTPB) have been implicated in the pathogenesis of COPD\[3\].

The matrix metallo protein family of enzymes consists of zinc dependent endoproteins in humans. The MMP3 gene is located on chromosome 11q.23. MMP3 (stromelysin) activates several other MMPs, and contributes to air way remodelling\[4\] to \[6\]. MMPs play a critical role in inflammatory airway diseases, tissue remodelling associated with various physiological and pathological processes such as morphogenesis, angiogenesis, tissue repair, and in regeneration of airway epithelium after injury and remodelling in COPD\[7\] to \[8\].

MMP3 cleaves collagen type III, IV, IX and degrades gelatine, fibronectin, laminin, elastin and proteoglycan link proteins\[9\] to \[10\]. It has been hypothesised that genetic variation affecting the expression of MMPs influences the development of COPD. The expression of MMPs in the lung is a highly regulated process and understanding its
regulation could in part shed light into their biological function in normal developmental process and in many polymorphic conditions\textsuperscript{[11-13]}. The studies on association of MMP3 gene variants with COPD are limited. Therefore, the aim of present study was to ascertain, if functional polymorphism in the promoter region of MMP3 is associated with the risk of developing COPD in a South Indian population from Andhra Pradesh.

**MATERIAL AND METHODS**

**Study population**

The Institutional ethical clearance was obtained to carry out the study. Special case proformas and consent forms for COPD and healthy controls subjects have been prepared to collect the detailed case histories and written consent from the cases willing to be recruited for the study had been taken.

A total of two hundred and fifty (n=250) COPD cases were taken from Government Chest Hospital, Irranuma, Hyderabad which is one of the reputed hospitals in Andhra Pradesh, where patients from different socioeconomic strata are referred. The cases which were diagnosed by spirometry, chest X-ray and confirmed by pulmonologists were considered for the study. The Spirometric classification of severity of COPD including four stages: stage I, mild; stage II, moderate; stage III, severe; stage IV, very severe COPD (cases with a history of cigarette smoking, cough, sputum, persistence dyspnea, acute exacerbations with their profession and other COPD risk factors were included for the study). Emphasis is given for the details of the epidemiological variables like age, sex, BMI, addictions such as smoking, Pack years in ex-smokers and other clinical profiles with physiological characteristics are shown in Table 1. An equal number of Clinically healthy controls and free of overt disease with same geographic background and similar socioeconomic status (n=250, mean age 41.79 ± 15.76 years; 9 female, 239 males, BMI 28.352 ± 8.014 kg/m2) were considered for the present study. (Table 1)

**TABLE 1 : Clinical and physiological characteristic of COPD group**

<table>
<thead>
<tr>
<th>Clinical and physiological parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>59.32 ± 10.29</td>
</tr>
<tr>
<td>Male/female, n</td>
<td>239/11</td>
</tr>
<tr>
<td>Body mass index, kg/m2</td>
<td>19.660 ± 4.990</td>
</tr>
<tr>
<td>Smoking status: smokers/ex-smokers/nonsmokers, n</td>
<td>197/45/09</td>
</tr>
<tr>
<td>Pack-years in smokers/exsmokers</td>
<td>88.2±19.768/75.8 ± 15.262</td>
</tr>
<tr>
<td>GOLD stage: I/II/III/IV, n</td>
<td>73/48/121/08</td>
</tr>
<tr>
<td>FVC, % predicted</td>
<td>78.122 ±10.196</td>
</tr>
<tr>
<td>FEV1, % predicted</td>
<td>42.102±10.962</td>
</tr>
<tr>
<td>FEV1/FVC, %</td>
<td>54.163±6.502</td>
</tr>
</tbody>
</table>

Values are n (%). *P value <0.05 considered as significant Data presented are mean value ±standard deviation (SD), number (%) of patients. BMI=body mass index. COPD=Chronic obstructive pulmonary disease.

**DNA analysis**

DNA was extracted from whole blood using salting out method (Lahiri et al)\textsuperscript{[14]}. The MMP-3 polymorphism was analysed by the polymerase chain reaction (PCR) with allele-specific primers (AS-PCR) using the following primers Forward 5’-GAT TAC AGA CAT GGG TCA CGG CAC-3’, reverse primer, 5’-AAT CAG GACAAG ACA TGG TTT TTC-3’ for the 5A allele and 5’-AAT CAG GAC ACA TGG TTT TTC-3’ for the 6A allele. Hot-start PCR was performed with the annealing temperatures being 65°C for the 5A allele and 62°C for the 6A allele, and 30 cycles of amplification were carried out. After amplification the PCR samples were loaded on 3% agarose gel and visualized under UV light in a gel documentation system. (Figure 1 Gel picture showing MMP3 5A/6A gene polymorphism)

**Statistical analysis**

The demographic characteristics like age, body
mass index, smoking, pack Years, were compared with the controls by student 't' test and Mann Whitney test. The 5A/6A polymorphism of MMP3 gene association between COPD and controls which was examined by Fischer exact ratio and chi-square analysis with 95% confidence interval (CI) and using Open EP16 software (Open Epi Version 2.3.1 from department of Epidemiology, Rollins school of Public Health, Emory University, Atlanta, GA 30322, USA). Genotypic frequencies were calculated according to the number of different genotypes observed and the total number of genotypes examined. Yate's correction (Yates 1934) was applied wherever necessary. Statistical significance was defined as p<0.05.

**RESULTS**

The MMP-3 5/6A polymorphism the genotypic frequencies of 5A/5A, 5A/6A and 6A/6A were 6.0%, 76.4%, and 17.6% in COPD patients, and 6.6%, 62.8%, and 24.0 respectively in controls (Table 2). The frequency of 5A/6A genotype was significantly high in COPD in comparison with controls (X=10.93, p=0.009, OR=1.91; 95% CI=13-2.828). However, there was no significant association found in allelic frequency in comparison with the control.

**DISCUSSION**

COPD is recognised as leading disease of lungs in many developed countries[15,16] MMPs and their potential effects in induction or progression of COPD constitute a recently developed field of research, with a number of observations supporting the role of MMP-1, MMP-9 and MMP-12[17]. Among these proteases, MMP-3 is considered a key enzyme, as it recognizes a broad spectrum of substrates, can activate other MMPs and it can be responsible for the release of growth factors[18]. In this study we have evaluated the role of matrix metalloproteinase (MMP3, stromolysin-1) 5A/6A polymorphism in the development of COPD. We analyzed two hundred fifty COPD patients with equal number of controls. The frequency of male COPD patients was found to be high compared to COPD females. Males are at higher risk for developing the disease in comparison to females because of addictions such as smoking[19] as females of our population are not addicted to smoking[20]. The higher preponderance of the disease was found in individuals with more than 45 years of age. This could be due to ageing where the lung functions get deteriorated leading to the susceptible development of COPD[21]. The frequency of smokers was also high in our patient group. The constituents of tobacco smoke diffuse across the alveolar capillary membrane and its lining fluid which enter into the blood circulation cause damage to endothelial cells[22,23].

BMI was found to be low in COPD patients when compared to healthy controls which is in accordance with previous studies suggesting low BMI as an important risk factor for COPD[24,25]. Although COPD is a lung related disease but the majority of its effects not only damage lungs but also show its effect in muscular weakness with a low body mass index (BMI)[26].

The 5A/6A of MMP-3 gene is a risk factor for susceptibility to COPD, since a significant difference was observed between patients and controls. To the best of our knowledge this is the initial study on MMP3 with 5A/6A genotyping which has been studied on COPD from South Indian population.

As this study shows that 5A/6A genotype a frequency was found to be high in COPD patients compared to controls. The mechanism behind the association of MMP3 metalloproteinases (MMP-s) are...
a feature of inflammatory conditions and may contribute to the overall evolution of the inflammation-induced tissue destruction[30]. Several pulmonary cells including resident alveolar macrophages, neutrophils, parenchymal cells (including interstitial fibroblasts), type II epithelial cells and vascular endothelial cells are capable of elaborating MMPs and numerous MMPs, including MMP3 and MMP9, have been considered to have important pro-inflammatory roles in acute lung inflammation[31].

MMP-3 plays different roles or a different role in maintaining the dynamic balance of the ECM, being responsible for cleavage activity on ECM components with potential release of growth factors (particularly those involved in fibrogenesis) and regulating the activity of a number of other MMPs. However, the study done so far on MMP-3 polymorphism in Caucasian Brazilian COPD patients indicates that there was no association of 5A/6A polymorphism with the disease[32]. Korytina and et al[33] studies have shown the association of MMP-3 gene with 6A/6A genotype[33]. The metaanalysis studies carried out by Hongbin Zhou and et al[34] have shown a moderate variation in 5A/6A MMP-3 gene polymorphism[34]. To confirm the association of MMP3 5A/6A gene polymorphism still there is a need to have further studies as MMPs are considered as candidate genes. The studies should also be carried out by metaanalysis on MMPs with COPD from India.

CONCLUSION

Our investigations support that MMP-3, 5A/6A genotype is a risk factor among COPD patients showing its effect on pathogenesis of COPD.

ACKNOWLEDGEMENTS

I would like to thank Professor and Director A.Jyothi, Faculty members of Department of cell biology, Department of Bio-chemistry, Institute of Genetics and Hospital for Genetic Diseases, Osmania University Hyderabad for guiding and suggesting for this work. We thank A.P.chest Hospital doctors, patients and staff for collaborating in this study. We thank UGC-MANF New Delhi for Financial aid.

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