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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 COPDfrom 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&MMP-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[4-6]. 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[7-8].

MMP3 cleaves collagen type III, IV, IX and degrades gelatine, fibronectin, laminin, elastin and proteoglycan link proteins[9-10] 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[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 excaberations with their profession and other COPD risk factors were included for the study). Emphasis is given for the details of the

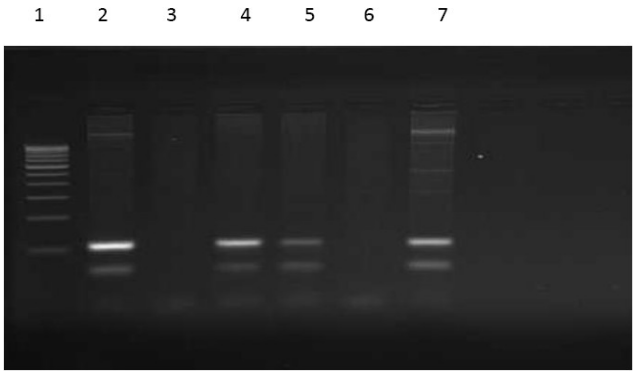

Figure 1 : Lane 1, 100bp DNA ladder, lane 2&3 homozygous 5A, lane 4&5, heterozygous 5A/6A, lane 6&7 homozygous 6A
Figure 1 : Agarose gel electrophoresis of PCR products bearing the MMP3 5A/6A polymorphism

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

Clinical and physiological parameters	Values
Age, years	59,32 ± 10,29
Male/female, n	239/11
Body mass index, kg/m2	19,660 ± 4,990
Smoking status: smokers/ex-smokers /nonsmokers, n	197/45/09
Pack-years in smokers/ exsmokers	88,2±19,768/75,8 ± 15,262
GOLD stage: I/II/III/IV, n	73/48/121/08
FVC,% predicted	78,122±10,196
FEV1, % predicted	42,102±10,962
FEV1/FVC, %	54,163± 6.502

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 etal)[14]. The MMP-3polymorphism 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 AAG ACA TGG TTT TTT-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

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TABLE 2 : Distribution of genotypes and allelic frequencies of MMP-3, 5A/6A gene polymorphism in COPD and controls

MMP-3	COPD patient		Controls	
5A/6A Genotypes	(n=250)		(n=250)	
Genotypes /Allele	NO	%	NO	%
5A5A	15	6	33	6.6
5A6A	191	76.4	15	62.8
6A6A	44	17.6	60	24
5A	221	4.2	23	44.6
6A	279	55.8	277	55.4

TABLE 3 : Comparison of genotypes and alleles of MMP-3 5A/6A in COPD and controls

Genotype/Allele	X^2	p-Value	odds Ratio 95% CI
5A5A	7.45	0.006	0.4197(0.2219-0.7941)
5A6A	10.93	0.009	1.91(13-2.828)
6A6A	3.1	0.07	0.6764(0.4373-1.046)
5A5A Vs 6A6	0.0016	0.898	0.9839 (0.7667-1.263)
6A6AVs 5A5A			1.016 (0.7919-1.304)

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 intial 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 compare 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 MMPsand 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 theECM, 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 polymor-phism in Caucasian Brazilian COPD patients indicates that there was no association of 5A/6A polymorphism with the disease[32]. Korytina and etal[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.

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REFERENCES

[1] C.J.Murray, A.D.Lopez; Global Burden of Disease Study. Lancet, **349(9064)**, 1498-504.1 **(1997)**.
[2] A.D.Lopez, K.Shibuya, C.Rao, C.D.Mathers, A.L.Hansell, L.S.Held et al.; Eur.Respir.J., **27(2)**, 397-412 **(2006)**.
[3] A.Nestor, M.D.Molfino; MSc, FCCPCHEST, **125**, 1929–1940 **(2004)**.
[4] V.Lagente, B.Manoury, S.Nénan, C.Le Quément, C.Martin-Chouly, E.Boichot; Brazilian Journal of Medical and Biological Research, **38**, 1521-1530 **(2005)**.
[5] F.Kheradmand, K.Rishi; New York: Dekker, 749–765 **(2003)**.
[6] J.J.Atkinson, K.Holmbeck, S.Yamada, H.Birkedal-Hansen, W.C.Parks, R.M.Senior; Dev.Dyn., **232**, 1079–1090 **(2005)**.
[7] D.A.Schwartz, D.S.Van Fossen, C.S.Davis, R.A.Helmers, C.S.Dayton, L.F.Burmeister, G.W.Hunninghake; Am.J.Respir.Crit.Care.Med., **149**, 444–449 **(1994)**.
[8] I.K.Demedts, G.G.Brusselle, K.R.Bracke, K.Y.Vermaelen, R.A.Pauwels; CurrOpin Pharmacol, **5**, 257–263 **(2005)**.
[9] K.Masumoto, J.D.de Rooij, S.Suita, R.Rottier, D.Tibboel, R.R.deKrijger; Histopathology, **47**, 410–419 **(2005)**.
[10] A.C.Newby; Physiol.Rev., **85**, 1–31 **(2005)**.
[11] C.Bergeron, M.K.Tulic, Q.Hamid; Eur.Respir.J., **29**, 596–604 **(2005)**.
[12] L.Joos, P.D.Pare, A.J.Sandford; Swiss.Med.Wkly., **132**, 27-37 **(2002)**
[13] S.R.Elshaw, N.Henderson, A.J.Knox, S.A.Watson et al.; Br.J.Pharmacol., **142**, 1318-1324 **(2004)**.
[14] D.K.Lahiri, J.I.Nurnberger Jr.; Nucleic Acids.Res., **19**, 5444 **(1991)**.
[15] D.M.Mannino, D.M.Homa, L.J.Akinbami, E.S.Ford, S.C.Redd; 1971–2000. MMWR Surveill Summ, **51**, 1–16 **(2002)**.
[16] C.J.Murray, A.D.Lopez; Science, **274**, 740–743 **(1996)**.
[17] Haq et al.; BMC Medical Genetic, **11**, 7 **(2010)**.
[18] A.M.Henney, P.R.Wakeley, M.J.Davies et al.; Proc.Natl.Acad.Sci., USA, **88**, 8154-8158 **(1991)**.
[19] D.James; Crapo PNAS.no.1, **(2010)**.
[20] C.Tabak, H.A.Smit, D.Heederik et al.; Clin.Exp. Allergy, **31**, 747–755 **(2001)**.
[21] K.Ito; **3**, 204–220 **(2001)**.
[22] R.S.Barua, J.A.Ambrose, L.J.Eales-Reynolds,

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M.C.DeVoe, J.G.Zervas, D.C.Saha; J.Am.Coll. Cardiol., **39**, 1758–1763 **(2002)**.

[23] R.S.Barua, J.A.Ambrose, S.Srivastava, M.C.DeVoe, L.J.Eales-Reynolds; Circulation, **107**, 2342–2347 **(2003)**.

[24] Arpana Vibhuti, Ehtesham Arif, Desh Deepak, Bhawani Singh, M.A.Qadar Pasha; Clinical Biochemistry, **40**, 958-963 **(2007)**

[25] M.I.Lewis, M.J.Belman; Clin.Chest.Med., **9**, 337-48 **(1988)**.

[26] D.O.Wilson, R.M.Rogers, E.C.Wright, N.R.Anthonisen; Am.Rev.Respir.Dis., **39**, 1435-8 **(1989)**.

[27] R.Naeije; Futura, **1992**, 209–239 **(1992)**.

[28] K.Nakano, T.Hiramoto, N.Kohno; Respir.Med., **97**, 915–920 **(2003)**.

[29] M.G.Belvisi, K.M.Bottomley; Inflamm.Res., **52**, 95-100 **(2003)**.

[30] H.Schirmer, L.Basso da Silva, P.J.Z.Teixeira, J.S.Moreira, A.L.S.Moreiraand, D.Simon; Genetics and Molecular Research, **8(3)**, 1028-1034 **(2009)**.

[31] D.F.Gibbs, T.P.Shanley, R.L.Warner, H.S.Murphy, J.Varani, K.J.Johnson; Am.J.Respir.Cell.Mol. Biol., **20**, 1145-1154 **(1999)**.

[32] P.Santus, F.Casanova, M.L.Biondi, F.Blasi, F.Di Marco, S.Centanni; Monaldi.Arch.Chest.Dis., **71(1)**, 15-20 **(2009)**.

[33] G.F.Korytina, O.S.Tselousova, L.Z.Akhmadishina, E.V.Victorova, Sh.Z.Zagidullin, T.V.Victorova; Mol.Biol. (Mosk). May-Jun, **46(3)**, 487-99 **(2012)**.

[34] Hongbin Zhou, Yinfang Wu, Yan Jin, Jiesen Zhou, Chao Zhang, Luanqing Che, Jiyong Jing, Zhihua Chen, Wen Li, Huahao Shen; Scientific Reports, **3**, 2818 **(2013)**.