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ORIGINAL ARTICLE

ASSOCIATION OF 1359 G/A POLYMORPHISM OF CNR1 GENE AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN PAKISTANI POPULATION

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ABSTRACT

Background: Chronic obstructive pulmonary disease (COPD) is slowly progressing respiratory disease that leads to alveoli blockage and difficulty in breathing. The objective of this study was to determine the association of 1359 G/A polymorphism of central cannabinoid receptor 1 **(**CNR1) gene and chronic obstructive pulmonary disease in Pakistani population.

Materials & Methods: We investigated CNR1 single nucleotide polymorphism at the location 1359 G/A (p.Thr453Th; rs1049353) in 180 COPD patients and 194 healthy individuals In Department of Biology, Virtual University, Lahore, Pakistan in 2014-2015. DNA samples were extracted from venous leukocytes by using standard method, amplified (PCR) and digested (RFLP). Restriction digests were evaluated by 8% polyacrylamide gel. Frequencies of homozygous and heterozygous variants of 1359 G/A polymorphism were analyzed.

Results: Genotype distribution AA, GG, GA of CNR1 gene in COPD patients was 1.1%, 68.3% and 30.5% versus controls 3.1%, 56.0% and 40.8% respectively. The heterozygous genotype distribution (GA) was observed higher in patients 68.3% than controls 56%. Allele frequencies A and G of CNR1 gene were 0.311 and 0.688 in controls versus 0.353 and 0.646 in patients respectively. The A allele variant frequency was higher in patients 35.3% than controls 31.1%. Results indicated that polymorphism of CNR1 gene located at 1359 may have association with COPD disease.

Conclusions: Our study gave clue for association of COPD with 1359 G/A polymorphism of central cannabinoid receptor 1 **(**CNR1) gene in Pakistani population. Further studies are needed to elucidate the relationship of CNR1 gene to COPD by larger sample sizes.

KEY WORDS: Cannabinoid Receptor; Genes; Genotype; Chronic Obstructive Pulmonary Disease; Polymorphism; Single Nucleotide Polymorphism; Pakistan.

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is slowly progressing chest ailment, in which lungs become damaged, making it difficult to breathe with the loss of the elastic quality of air sacs "alveoli".[1] Other specifications of COPD include alveoli-destruction; also known as emphysema and chronic bronchitis in the advanced phase of the disease. The chronic bronchitis is the production of sputum and cough in consecutive two years for more than three successive months and is not necessarily linked with air flow limitation.[2]

The lipid-derived system of signaling known as endo-cannabinoid system is involved in many physiological functions of the body including inflammatory processes, energy metabolism and immune responses. The system contains two genes CNR1 and CNR2 which are encoded by two G-coupled receptors CNR1 and CNR2 respectively.[3]

CNR1 is abundantly expressed in the brain mainly in hippocampus and cerebellum[4] and is also found in lungs, testis, ovaries, liver, muscles, heart, spleen, pancreas, gastrointestinal tract and kidneys.[5] It is

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also involved in mediating the hypothermic, hypolocomotive and hypotensive effects of cannabinoids; whereas CNR2 gene is expressed in the hematopoietic stem cells, tonsils, gastrointestinal tract and immune system.[6]

COPD is connected to the uncharacteristic inflammatory reactions exhibited by the lungs to smoke, dust and noxious chemicals. It is an avertible and curable disease with some significant extra-pulmonary outcomes that boost disease severity.[7] Prominent problem for annihilation and inflammation of small airways is the irreversible enlargement of small distal air spaces. So both the abnormalities of air spaces and airways (small and central) are present in patients of COPD.[8] The common symptoms for COPD disease include production of sputum, coughing and breath-difficulty.[9] The utmost cause for exacerbation is air-pollution and the infection in bronchial tree of trachea. According to WHO reports for 2030, COPD disease is prophesied to become 3rd leading reason for causalities.[10] It is frequent in the aged people (more than 75 years). Globally the prevalence of COPD is 9-10% in the individuals aged ≥ 40.[11]

There are many environmental and genetic factors involved in COPD. Smoking tobacco is the major environmental factor which is causing COPD.[12] Others include; industrial exposure to fume and dust, air pollution, prior tuberculosis and inhalation of biomass smoke.[13,14]

The gene CNR1 is localized in q14–q15 section of chromosome 6 in humans. Different polymorphisms are studied in CNR1 gene.[20] The CNR1 gene polymorphism (1359 G/A) is the result of guanine to adenine's exchange at location 1359 in codon 435 (Thr).[21]

Frequent studies have shown the association of CNR1 gene with various diseases.[3,22] But there is no data found on COPD disease in relation to CNR1 gene polymorphism. So we have selected this gene polymorphism in the current study. The objective of this enquiry was to explore the association of 1359 G/A polymorphism of central cannabinoid receptor 1 **(**CNR1) gene and chronic obstructive pulmonary disease in Pakistani population.

MATERIALS AND METHODS

Design, setting & duration: This comparative cross-sectional study was conducted at the Department of Biology, Virtual University, Lahore, Pakistan in 2014-2015. The blood samples were collected from Jan. 2006 to Feb. 2008 and the laboratory analysis of preserved DNA was done in 2014-2015.

Subjects: Two samples; one of 180 COPD male patients and other of 194 healthy subjects were included in this study. Consultant pulmonologist at Sheikh Zayad Hospital and Gulab Devi Chest Hospital, Lahore, Pakistan confirmed the diagnosis of patients and controls by clinical evaluation and pulmonary function tests. Those with any acute or chronic diseases were excluded. Written informed consents were signed from all the willing participants.

Detection of the gene CNR1 allele and its genotypes: The DNA was isolated from the preserved blood samples by the use of standard salting out methods.[23] Genomic DNA sequence of 111bp of the 5′ section of CNR1 was enlarged by use of primer pair 5′- GAAAGCTGCATCAAGAGCCC-3' and 5′-TTTTCCTGTGCTGCCAGGG-3'.[21]

PCR mixture of 20 μl contained 7 μl of the PCR water (GeNei™), 9.8 µl of master mix, 1 µl of both primers (forward and reverse), 0.2 µl of DNA Taq polymerase and 1 µl of genomic DNA (Vivantis, 2010, Malaysia). PCR intensification conditions were; for 1st step at 94°C for 10 seconds, followed by 30 denaturation cycles at 94°C for 10 sec/ cycle, 2nd step by annealing at 60°C for 5 seconds, 3rd step by extension for 15 sec at 72°C and last step of final extension at 72°C for one minute. It yielded PCR product of 111 bp amplicon which was then digested by enzyme *MspI* (TaKaRa, Otsu, Shiga, Japan) for 16 hours at 37°C. Afterwards, the products of PCR were separated by 8% polyacrylamide gel electrophoresis (PAGE) that was stained by using ethidium bromide dye and was visualized on an UV fluorescence imaging system. (Figure 1 and 2)

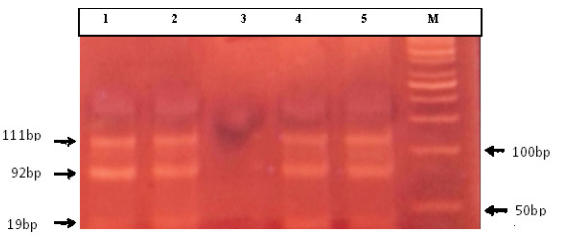

Figure 1: Lane 1 & 2; heterozygous genotype (GA) of patients, Lane 3; doesn't contain any representative sample, Lane 4 & 5; heterozygous genotype (GA) of normal samples, M: ladder of 50bp

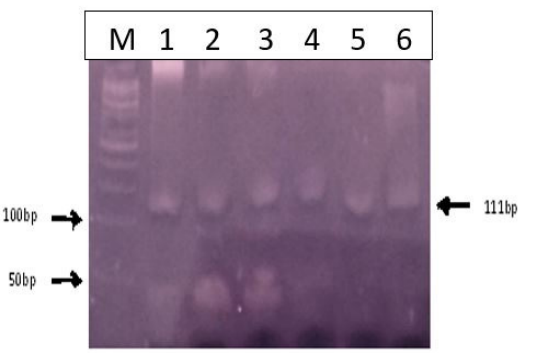

Figure 2: M; ladder of 50bp, Lane 1, 2 & 3; heterozygous genotype (GA), Lane 4, 5 & 6; undigested homozygous genotype (AA)

RESULTS

We aimed to study polymorphism of CNR1 gene in association with COPD disease in Pakistani population. The homozygous and heterozygous frequency values were calculated. The genotype distribution AA, GG, GA of CNR1 gene in COPD patients were 1.1%, 68.3%, 30.5% in comparision with controls which was 3.1%, 56.0%, 40.8% respectively. The heterozygous genotype distribution (GA) was observed higher in patients (68.3%) than in controls (56%). The allele frequencies A and G of CNR1 gene were 0.311 and 0.688 in normal individuals as compared to 0.353 and 0.646 in patients respectively. The A allele variant frequency was higher in patients (35.3%) as compared to control (31.1%).

DISSCUSSION

COPD damages the respiratory tract and make it hard to breath. It destroys the lungs and causes dyspnea and elasticity of air sacs become lesser. It is a process of successive cough for years producing sputum and wheezing. One of the most common causes of COPD is smoking tobacco. It is frequent in the aged people.[24]

In SAARC countries (India, Nepal, Bhutan, Bangladesh, Sri Lanka, Afghanistan, Maldives and Pakistan), COPD is a common cause of death. The prevalence of COPD in Indian population more than the age of 35 is 3.49% and the national burden was estimated to be 14.84 million cases.[25] In Bangladeshian population, COPD is distributed by sex as 76.9% in men and 23.1% in women.[13] In Nepal due to smoking tobacco, prevalence of COPD was estimated to be 7.7% higher in men as compared to women.[26] In Sri Lankan population, COPD prevalence is also high.[27] In Bhutan, Maldives and Afghanistan no study is available on COPD prevalence.

In Pakistan higher prevalence of COPD is reported.[28] Pakistan has the 4th highest mortality rate from COPD among the 25 most populous countries in the world, and the estimated mortality rate by COPD in Pakistan is 71 deaths per 100,000.[29]

CNR1 gene in the brain is involved in mediating the hypothermic, hypolocomotive and hypotensive effects of cannabinoids.[30] Different countries in the world have reported association of CNR1 gene with several other diseases such as Japan and France on schizophrenia,[31,32] Brazil on Obesity,[33] Turkey on cannabis addiction,[34] Germany on Crohn`s Disease[35] and Spain on diabetes mellitus 2 & obesity.[36] But there is no research work reported on CNR1 gene in relation to COPD disease.

Currently no research work has been reported on 1359 G/A polymorphism of CNR1 gene from SAARC countries (Afghanistan, India, Bangladesh, Sri Lanka, Bhutan, Maldives and Nepal). Nearest data has been reported from Turkey,[34] whose results are similar to our study results. In the Pakistani population, CNR1 gene 1359 SNP research has been reported in few studies which have linked this SNP to schizophrenia.[22] At present no advance data has been reported on this SNP.

Currently there is no data on COPD disease in relation to CNR1 gene polymorphism in Pakistani population. We investigated CNR1 gene 1359 G/A polymorphism in 180 COPD male patients and 194 healthy subjects. The heterozygous genotype distribution (GA) was observd higher in patients (68.3%) than in controls (56%) which is similar to studies reported from Turkey.[34]

Our results show the allele frequency of G allele (68.6%) is higher than the frequency of A allele (31.1%) which is similar to the previous studies.[37] The current study shows same increase in the allele frequency of G (68.8%) in controls than the patients (64.6%) as shown by other studies.[32,34,37,38] The A allele variant frequency was higher in patients (35.3%) as compared to controls (31.1%); is similar to the reported findings.[39,40] This is the first research work reported on COPD disease in relation to CNR1 gene 1359 G/A polymorphism in Pakistan.

CONCLUSIONS

Our study gave clue for association of chronic obstructive pulmonary disease (COPD) with 1359 G/A polymorphism of central cannabinoid receptor 1 **(**CNR1) gene in Pakistani population. Further studies are needed to elucidate the relationship of CNR1 gene to COPD by larger sample sizes.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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None declared.

AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	IA, MAK
Acquisition, Analysis or Interpretation of Data:	IA, MAK, SA
Manuscript Writing & Approval:	IA, MAK, SA

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.