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Association of *Aquaporin 5* (*AQP5)* gene variant rs146762965 with chronic obstructive pulmonary disease (COPD) in North Indian population

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is a type of obstructive lung disease characterized by persistent airflow limitation. It is preventable, treatable but progressive and associated with co-morbidities. According to WHO, COPD presently is the third leading cause of death in the world and is expected to rank second by 2020. Aquaporin 5 (AQP5) is a membrane channel protein playing an important role in fluid secretion, altered regulation of which results in pulmonary diseases. AQP5 gene is located on chromosome 12q13 and the single nucleotide polymorphism (SNP) rs146762965 lies in the coding region corresponding to the secondary structure of channel protein. Our objective was to evaluate the association of this polymorphism with COPD in north Indian population. Genotyping was successfully done in controls and cases (100 each) by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR products were digested with restriction enzyme (FauI) and analyzed on 12% polyacrylamide gel (PAGE). Allele and genotype frequencies in both the groups were compared statistically by SPSS (v21.0). Genotypic frequency showed 19% CC and 81% CT in patients, while 42% CC and 58% CT in controls. The allelic frequency showed 71% of 'C' allele in controls as compared to 59.5% in patients. The 'T' allele was 29% in controls and 40.5% in cases. Due to the higher frequencies of 'CT' genotype in COPD cases, there is a significant association (P<0.01) with the disease. The 'T' of rs146762965 polymorphism may be the risk allele for COPD in the North Indian population.

Key words: COPD, AQP5, Single Nucleotide Polymorphism, PCR-RFLP

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a type of lung disease characterized by persistent airflow limitation as proposed by Global initiative for chronic Obstructive Lung Disease (GOLD) [1]. It is preventable, treatable but progressive and associated with co-morbidities. In a recent update by World Health Organization [2], COPD has become the 3rd leading cause of death and is estimated to be second by 2020. Half a million people in India die every year due to COPD which is four times higher than Europe and America [3]. Smoking is the main causative agent; others include exposure to biomass (wood, animal dung burning stoves), fumes and dust during work. Although smoking is the main cause of COPD but only 15% smokers develop the disease [4]. This inter-individual difference implies that genetics plays an important role in disease manifestation. The irreversible airflow limitation occurs due to chronic inflammation and altered mucous production [5;6]. Severity of airflow limitation is categorized into four groups by GOLD based on post bronchodilator FEV_1 (Forced Expiratory Volume in one second).

AQP5 is one of the members of aquaporin family which are water specific membrane channel proteins [7]. They are found in airway epithelial cells, type I alveolar epithelial cells and submucosal gland acinar cells in the lungs. AQP5 consists of six transmembrane domains connected by five loops [8]. Its altered regulation results in impaired fluid secretion in pulmonary diseases [9]. Lowered lung function and mucous overproduction in COPD is associated with decreased expression of AQP5. In addition, cigarette smoke also decreases the expression of *AQP5* in submucosal gland [10].

AQP5 gene located on chromosome 12q13 has 4 exons and 3 introns [11]. The change in the allele from 'C' to 'T' in C/T polymorphism (rs146762965) corresponds to a change in amino acid from arginine to cytosine in the exonic region which encodes the secondary structure (turn) of AQP5 protein. As the turn is present in the exposed portion of the protein, the change in polarity of the residue alters the function of the protein. The aim of the present study was to evaluate the association of this polymorphism with COPD in north Indian population.

MATERIALS AND METHODS

Patient selection and clinical evaluation

COPD patients (n=100) were enrolled from the outpatient clinic of Department of Pulmonary Medicine, King George's Medical University (KGMU), Lucknow, India under the supervision of expert clinicians. Age and sex matched healthy individuals were included in the study from staff members of both universities. The study was approved by Institutional Ethics Committee (2609/R-Cell-12 dated 20.10.2012) and a written consent was taken from all subjects. Controls with no history of respiratory, cardiac or any other disease were included. Clinical identification of patients was done according to the GOLD criteria which included cough, chest pain, exacerbation, breathlessness, *etc*. Blood samples (2 ml) were collected in EDTA vials and stored at -20^{0}C until further use.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using salting out method [12] with slight modifications [13]. Genotyping of *AQP5* C/T polymorphism (rs146762965) was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism analysis (PCR–RFLP). The 15 µl reaction mixture contained 100 ng of template DNA, buffer (100 mM Tris, pH 9.0; 500 mM KCl; 15 mM $MgCl_2$; 0.1% gelatin), 200 µM dNTP, 10 pmol of each primer and 1.0 unit Taq DNA polymerase. The primers designed by Primer 3.0 online software were F: 5'-GGTGGAGCTGATTCTGACCT-3' and R: 5'-ATCCATCTGAAGTGGGCTCC-3'. The PCR products were digested with FauI restriction enzyme (New England Biolabs, USA) and electrophoresed on 12% polyacrylamide gels.

Statistical Analysis

Allele and genotype frequencies were compared using Chi square test ($\chi 2$) and Fisher's exact t-test. All P values were two-sided and differences were considered statistically significant for $P<0.05$. Odds ratio (OR) at 95 % confidence intervals (CI) was determined to describe the strength of association by Logistic Regression Model.

RESULTS

The *AQP5* C/T gene polymorphism was successfully genotyped in controls and COPD cases (n=100 each). The allele and genotype frequency distributions are shown in Table I. Individuals with genotypes 'CC' and 'CT' were found in the study population (Fig. I.)

Allele and genotype frequencies in cases and controls were compared statistically by SPSS (v21.0). Among COPD cases, 19% were CC and 81% CT while in controls 42% were CC and 58% CT. The allelic frequency showed 71% of 'C' allele in controls as compared to 59.5% in cases. The 'T' allele was 29% in controls and 40.5% in cases. No individuals with 'TT' was observed in our population (Table I). On comparison, both genotypic and allelic frequencies showed significant association with the disease ($P<0.0001$, <0.016) (Table I). The odds ratio (OR) in case of 'T' allele was >1.5.

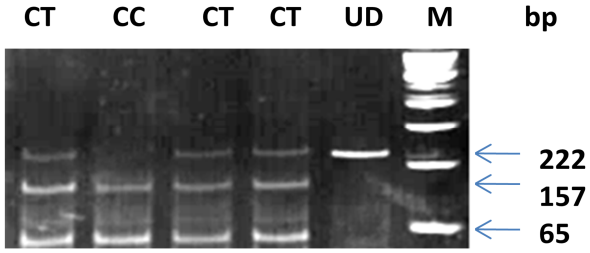

Fig. I. 12% PAGE showing *AQP5*C/T polymorphism (rs146762965) using FauI restriction enzyme. UD: undigested; M: 100 bp DNA Ladder

Table I: Genotypic and allelic frequencies of *AQP5* C/T polymorphism in healthy controls (n = 100) and COPD cases (n = 100).

Genotype Frequency	Number (%frequency)				P value
		CC	**CT**	**TT**	
	Controls	42(42.0)	58(58.0)	00	
	Cases	19(19.0)	81(81.0)	00	**<0.0001**

Allele Frequency	Number (%frequency)			P value	Odds ratio	%95 CI
		C	**T**			
	Controls	142(71.0)	58(29.0)			
	Cases	119(59.5)	81(40.5)	**<0.016**	1.666	1.099-2.526

DISCUSSION

There has been evidence that *AQP5* expression in lungs has a key role in regulation of mucous and fluid secretion by submucosal glands [9;14]. Mucous is an important component of lung's defense mechanism but its abnormal secretion may lead to dysfunction of antibiosis enzymes and antibacterial peptides [15]. Wang *et al* [14] have shown that expression of *AQP5* is related to severity of airflow obstruction in COPD patients. The association between the *AQP5* polymorphisms and COPD risk was evident among the Chinese population [16].

The association of *AQP5* C/T polymorphism with COPD was studied for the first time in the north Indian population but no definitive conclusion could be drawn. This was due to the fact that the study population was not in Hardy-Weinberg equilibrium at this locus. Individuals of only two genotypes, one homozygous 'CC' and the other heterozygous 'CT' were found. A significantly higher frequency of COPD patients appeared to have 'CT' genotype. This was also evident from the presence of significantly higher frequency of 'T' allele in cases. Although the minor allele frequency (MAF) was below 0.01, the fact that the presence of 'T' allele was higher in COPD cases cannot be overlooked. According to this preliminary result 'T' can be attributed as the risk allele in the study population. The limitation of the study however is the less number of subjects. It would be worthwhile to increase the sample size in order to confirm the SNP association.

In conclusion, this study suggests that since the 'T' allele of *AQP5* C/T polymorphism was more prevalent in COPD cases, it seems to be associated with COPD while 'C' allele seems to be protective in the north Indian population. Such genetic studies will help to find prognostic markers for determining disease susceptibility in different populations.

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