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The Study of the Association in between CHRNA 5 Gene Polymorphism (rs16969968) and Chronic Obstructive Pulmonary Disease

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Abstract

Background and Aim: Chronic obstructive pulmonary disease (COPD) is a complex, progressive inflammatory disorder of the respiratory system that predominantly affects the distal airways and is characterized by irreversible destruction of lung parenchyma. Genetic susceptibility plays an important role in the pathogenesis of COPD. The objective of this study was to examine the relationship between COPD and *CHRNA5* gene polymorphism (rs16969968).

Methods: A total of 200 subjects were enrolled, including 100 diagnosed COPD cases and 100 age- and sex-matched healthy controls. Genotyping of the single nucleotide polymorphism (SNP) rs16969968 was carried out using allele-specific polymerase chain reaction (AS-PCR) with specific primers. Allele and genotype frequencies were measured, and statistical analysis was conducted using SPSS software to assess the association between *CHRNA5* gene polymorphism and COPD risk.

Results: The distribution of *CHRNA5* (rs16969968) genotypes did not show a statistically significant association with COPD. The AA genotype was observed in 6% of controls and 15% of COPD cases, the heterozygous AG genotype in 39% of controls and 43% of COPD patients, and the homozygous GG genotype in 55% of controls and 42% of COPD cases. The wild-type G allele was found in 149 (74.5%) of controls and 127 (63.5%) of COPD patients, while the A allele was found in 51 (25.5%) of controls and 73 (36.5%) of COPD cases. Statistical analysis revealed no significant difference in allele frequencies ($p = 0.157$). Although genotype distribution showed a trend toward significance ($p = 0.055$), it did not reach the conventional threshold ($p < 0.05$). Odds ratio analysis revealed an OR of 1.688 ($p = 0.066$), suggesting a possible increased risk among A allele carriers.

Conclusion: This study did not demonstrate association between the CHRNA5 rs16969968 polymorphism and COPD. However, odds ratio analysis indicated a possible 1.7-fold increased risk of COPD among carriers of the A allele, suggesting a potential role of CHRNA5 variants in disease susceptibility. Larger studies with diverse populations are warranted to further clarify the contribution of CHRNA5 variants to COPD risk.

Keywords: Chronic Obstructive Pulmonary Disease; Polymorphism; Single Nucleotide, Genotype; Alleles; Smoking; Tuberculosis; Polymerase Chain Reaction.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease presenting with symptoms of lung illness as well as airflow restriction. COPD refers to a group of conditions, primarily chronic bronchitis and emphysema, associated with irreversible expiratory airflow obstruction, (1,2). In the United States, COPD is the fourth leading cause of death, following cardiovascular disease, cancer, and cerebrovascular disorders (3, 4). The major risk factor is cigarette smoking, which is largely preventable. Majority of patients are of middle-aged or older adults. Approximately 16 million outpatient visits in 2000 were attributed to conditions associated with COPD (5). Yet, not all cigarette smokers have COPD, indicating that genetic factors have a role in disease susceptibility. Inflammation of the lungs and air passageways of the bronchi caused by cigarette smoking or environmental toxins leads to parenchymal damage and small airway disease (6, 7). Genetics contribute largely to the development of COPD among smokers. Studies conducted on families as well as twins indicate the aggregation of COPD within families, estimating that genetic components account for between 30-60 percent of COPD risk (8). Genetics and smoking act synergistically as risk enhancers for COPD among certain individuals. There are certain genetic predispositions which make the lungs vulnerable to the adverse effects of smoking. Hence, individuals with a high genetic predisposition are more likely to develop COPD compared with those having a low genetic predisposition, even when smoking the same amount.

Research indicates that individuals with both high genetic risk levels and active smoking behavior have more than 11 times the risk of COPD compared with those with low genetic risk levels who never smoked. Some genes that determine how well the lungs are able to handle smoking-induced damage and inflammation can predispose individuals to COPD. For example, those with gene variations in the *CHRNA5* gene have higher complications of COPD if they are smokers as their lungs lack adequate safeguards against the effects of the chemicals found in tobacco. The nicotinic acetylcholine receptor alpha5 subunit gene (*CHRNA5*) has been extensively implicated in COPD susceptibility. The most implicated variant in this gene is the rs16969968 single nucleotide polymorphism (SNP) that affects the protein altering its structure. Research indicates that the gene is associated with the risk of COPD in multiple populations. Individuals with the rs16969968 gene variant show higher susceptibility rates of COPD, with higher odd ratios across different genetic models. Although genetic risk exists independently of smoking in most populations, the effects of CHRNA5 variants are more pronounced among smokers due to their role in inflammatory conditions and impaired lung remodeling without proper repairs (9).

2. Methods

Study Design and Participants

This case-control study was conducted in the Department of Biochemistry, Santosh Deemed to be University, Ghaziabad, Delhi NCR. COPD patients were recruited from the Outpatient Department (OPD) of Respiratory, Santosh Hospital, Ghaziabad, Delhi NCR. Written informed Consent was obtained from all COPD cases and age-and sex-matched healthy controls who voluntarily agreed to participate in the study.

Inclusion Criteria

- Clinically diagnosed COPD patients aged above 30 years
- Diagnosis of COPD was established by a clinician based on:
    - Detailed medical history
    - Physical examination
    - Laboratory investigations
    - Pulmonary function testing (spirometry)
    - Chest X-ray
    - Special investigations where indicated, including fiberoptic bronchoscopy

Exclusion Criteria

- Age less than 30 years
- History of tuberculosis
- Lung cancer
- Pulmonary fibrosis
- Endocrine disorders
- Hematological diseases
- Liver disease
- Heart disease
- Any other malignancy

Sample Collection

Venous blood sample were collected from both patients and healthy controls via the cubital vein under strict with all aseptic precautions, using Vacutainer and Needle. 2.0 ml was collected in the BD vacutainer with EDTA as anticoagulant and stored at -20^0C in two aliquots of 1 ml each for DNA extraction.

Polymerase Chain Reaction (PCR) of CHRNA5 Gene

Table 1. Primers for Allele-Specific Polymerase Chain Reaction (AS PCR)

SNP	PRIMER SEQUENCE (5'-3')	ALLELE	Tm	Ta	AMPLICON SIZE
rs16969968 (G>A)	FORWARD PRIMER (FP): CGC TAT CAA CAT TCA TCA TC		53.2	55.6	
	REVERSE ALLELE SPECIFIC PRIMER (RPA): CTT GTA ATG TAG CGA ATA GAA GT	A	55.3		215
	REVERSE ALLELE SPECIFIC PRIMER (RPG): CTT GTA ATG TAG CGA ATA GAA GC	G	57.08		215

Table 2. Contents of standardized Polymerase Chain Reaction (PCR) reaction mixture (10 µl) for CHRNA 5 Gene

S. No.	Components	Volume Tube 1	Volume Tube 2
1.	Master Mix	5µl	5µl
2.	Primers (FP)	0.3µl	0.3µl
3.	RPA	0.3 µl	0 µl
4.	RPG	0 µl	0.3 µl
5.	DNA	2µl	2µl
6.	Nuclease Free Water	2.7µl	2.7µl
	Total volume	10µl	10µl

Polymerase Chain Reaction (PCR) and Genotyping

Table 2 shows the ingredients used to prepare two tubes for a PCR experiment. Each tube contained 5 µl of Master Mix (DXBIDT, Bengaluru, Karnataka, India), 0.3 µl of forward primers, 2 µl of DNA, and 2.7 µl of nuclease-free water, making a total volume of 10 µl. Tube 1 contained 0.3 µl of RPA but no RPG, while Tube 2 contained 0.3 µl of RPG but no RPA. This setup allowed for testing different reactions by slightly changing one component in each tube.

The PCR mixture was prepared in the 200 µl tube, which was then left inside the thermocycler (Real-Time System, Bio-Rad). To amplify the target DNA molecule, the process of PCR was employed. The amplified product of the PCR reaction was then kept at 4°C until the beginning of the onset of the electrophoresis of PCR. The PCR procedure began with the initial phase called 'Denaturation,' in which the mixture was exposed to the temperature of 95°C for 5 minutes and then both strands of the double-stranded DNA were completely separated into single strands. Next came the 'Cycle' phase, in which a cycle consisting of three stages was repeatedly executed for a total of 35 times. The first stage was 'Denaturation', where a temperature of 95°C was applied to the mixture for a period of 30 seconds and both strands maintained separated. The next stage was 'Annealing,' where the mixture was cooled to a temperature range of 55-60°C for a period of 30 seconds, during which the primers attached to the target molecule. The final stage was 'Extension,' where a temperature of 72°C was applied to the mixture for a period of 30 seconds. Following the entire procedure, a final 'Extension' was done at a temperature of 72°C for a period of 3 minutes, during which any incomplete strands were entirely extended. The reaction then stoped at a temperature of 4°C for an infinite period until the end product was collected. Subsequently, the PCR product and DNA ladder were added to the wells, then the unit was attached to the power source and the electrophoresis was done at a constant voltage of 100V. Once the dye had covered two-third of the distance, the power source was switched off. Finally, the PCR product was analyzed using a gel documentation system.

3. Results

Genomic DNA extracted from the whole blood by the modified manual method was typically about 24kb in size (Figure 1) and was suitable for carrying out AS PCR.

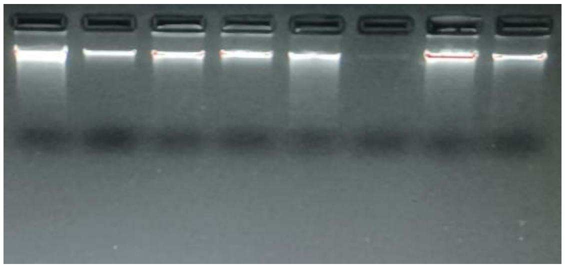

Figure 1. Result of manually extracted DNA

After the DNA extraction, the desired section of the COPD gene was amplified using PCR with specific forward and reverse primers (Table 1). Band of around 215 bps was obtained as a PCR product after the Agarose Gel documentation system analysis and gel electrophoresis (Figure 1).

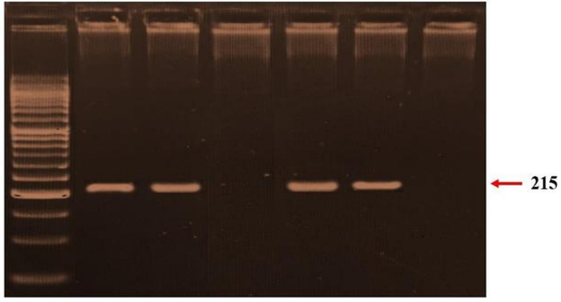

Figure 2. AS PCR result after the agarose analysis and gel electrophoresis employing a gel documentation system. Lane 1 M- DNA marker; Lane 2 – PCR product, forward primer + A-allele reverse primer; Lane 3- PCR product forward primer + G-allele reverse primer band size (215bp) heterozygous (AG) genotype; Lane 4- PCR product, forward primer + A allele reverse primer; Lane 5- PCR product, Forward primer + G allele reverse primer band size (215bp) homozygous (AA) genotype; Lane 6- PCR product, forward primer + A allele reverse primer; Lane 7-PCR product, Forward primer + G allele reverse primer band size (215bp) homozygous (GG) genotype.

For genotyping of *CHRNA5* (rs16969968), a single forward primer and two reverse primers specific to the A and G alleles were used respectively. With the two reverse primers used in the study, it was possible to distinguish between the two variants (A and G) of the particular gene sequence. PCR fragment size produced with the combinations of primers was 215bp and was analyzed using agarose gel electrophoresis.

Statistical analysis

The statistical analysis was conducted using SPSS (Software 17). The calculation of frequency of alleles in both COPD cases and controls was carried out as demonstrated in table no 3. The AA (Homozygous) genotype of the *CHRNA5* gene was found in 6 (6%) of controls and 15 (15%) of COPD patients; the AG (Heterozygous) genotype was found in 39 (39%) of controls and 43 (43%) of COPD patients; the GG (Homozygous) genotype was found 55 (55%) in control populations and was absent 0 (0%) among COPD patients. The wild-type G alleles were found in 149 (74.5%) of control and 127 (63.5%) of COPD cases; The A alleles were found 51 (25.5%) in controls and 73 (63.5%) in COPD cases. The results of the statistical analysis showed that there were no significant variations among alleles (p=0.157); however, there were variations among genotypes which were found close to significance (p=0.055). Nevertheless, p value did not reach the conventional threshold (p<0.05). Calculation of OR was done through crosstabs with OR= 1.688 and p values (p=0.066) as demonstrated in Table 4.

Table 3. Allele frequency and genotype in COPD patients and the control group

			Genotype				**Allele**	
	N	**AA(%)**	**AG(%)**	**GG(%)**	**P-value**	**A(%)**	**G(%)**	**P-value**
Control	100	6 (6)	39 (39)	55 (55)		51 (25.5)	149 (74.5)	

COPD	100	15 (15)	43 (43)	42 (42)	.055	73 (36.5)	127 (63.5)	.157

Table 4. CHRNA5 A-Allele result in COPD patients and control group

		COPD (n=100)	**Control (n=100)**	**P-value**	**OR**	**95% Class Interval**	
						Lower	**Upper**
CHRNA5 A-Allele	Present	58	45	0.066	1.688	0.965	2.953
	Absent	42	55				

4. Discussion

Chronic obstructive pulmonary disease (COPD) is a progressive respiratory disorder characterized by persistent airflow limitation and chronic inflammatory responses affecting the airways and lung parenchyma. COPD is primarily caused by prolonged exposure to harmful particles or gases, most frequently given off by cigarette smoke (10,11). COPD is one of the leading causes of morbidity and mortality globally, ranking among the leading contributors to global disease burden and healthcare costs (12,13). The pathogenesis of COPD is complex and multifactorial, involving an interaction between environmental exposures, such as tobacco smoke, biomass fuel, and occupational pollutants, as well as genetic factors which modulate vulnerability of an individual(14,15). While environmental risk factors are rather well recognized, genetic predisposition, including polymorphisms in genes regulating inflammatory processes, oxidative stress responses, and lung tissue remodeling, may have a significant impact on COPD risk, progression, and severity.(16,17). Among these, variants in the *CHRNA5* gene have drawn considerable attention for their potential role in nicotine dependence, airway inflammation, and lung function decline (18,19)

In the present study, we examined the association between the *CHRNA5* rs16969968 polymorphism and COPD by comparing the frequency of genotype and allele between COPD cases and healthy controls. The AA genotype was more frequent among COPD cases (15%) compared to controls (6%), while the GG genotype was less frequent in COPD cases (42%) compared to controls (55%). Similarly, the A allele frequency was higher in COPD patients (36.5%) compared to controls (25.5%). Although these differences suggested a possible association, statistical analysis did not reveal a significant difference in allele frequencies (p = 0.157). However, the distribution of genotypes presented a trend of significance (p = 0.055). Odds ratio analysis indicated a potential 1.7-fold increased chance of developing COPD among A allele carriers; nonetheless, this association was not statistically significant (OR = 1.688, 95% CI = 0.965–2.953, p = 0.066). These findings are consistent with previous studies showing an implication of CHRNA5 gene polymorphism in COPD susceptibility (20). The CHRNA5 gene encodes the α5 subunit of the nicotinic acetylcholine receptor, which is implicated in regulating nicotine dependence, airway inflammatory responses, and respiratory function. Some polymorphisms, especially rs16969968 and its correlated variants, have been reported to increase the risk of COPD and other smoking-related respiratory diseases in various populations (21-23). However, in line with our findings, several other studies have reported non-significant associations, often attributed to small sample sizes, population heterogeneity, or other various environmental exposures such as smoking intensity and occupational hazards (24).

In this study, the observed trend toward the higher prevalence of the AA genotype and A allele among COPD patients suggests that CHRNA5 polymorphism may contribute to disease risk. Previous studies have proposed that the A allele may reduce receptor efficiency and impair cholinergic signaling, potentially intensifying airway inflammation and lung tissue damage. Nevertheless, the lack of statistical significance in our study highlights the need for larger, multi-center studies across diverse populations, with control for environmental factors, to further clarify this association(25).

In summary, while our findings point to a possible link between the AA genotype of the CHRNA5 gene and increased susceptibility to COPD, the lack of statistical significance limits definitive conclusions. Future research with larger cohorts and detailed analysis of gene– environment interactions could provide more robust evidence regarding the role of CHRNA5 variations in the physiology of COPD.

5. Conclusion

The present study investigated the association between CHRNA5 gene polymorphism (rs16969968) and susceptibility to COPD. Although the AA genotype and A allele were more frequently observed among COPD patients compared to healthy controls, no statistically significant differences were identified in genotype or allele distributions. However, the odds ratio analysis showed the potential 1.7-fold increased risk for COPD due to the A allele, implying that polymorphism at the *CHRNA5* gene may be responsible for the disease susceptibility. Given the lack of statistical significance, these results should be interpreted with caution. They tend to support the hypothesis that genetic factors, in addition to environmental factors, play a role in COPD development. Future studies with larger sample sizes, diverse populations, and comprehensive evaluation of gene–environment interactions are warranted to better elucidate the contribution of *CHRNA5* polymorphisms to COPD development and progression.

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Ethical Considerations and Compliance with Ethical Guidelines

Study protocol was approved by Institutional Ethics Committee and the written informed consent was obtained from study participants or legal guardians. The study conducted in accordance with the principles of the Declaration of Helsinki.

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Conflict of interest

On behalf of all authors, the corresponding author declares that there is no conflict of interest.

AI Using Declaration

The authors declare no artificial intelligent chatbot use.

Author's contributions

All authors contributed equally to the conception; design; data collection; analysis, and preparation of the manuscript.

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