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RESEARCH ARTICLE

Investigation of the Relationship between the Multidrug Resistance 1 Gene Polymorphisms and Bronchodilator Response in COPD

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ABSTRACT

Objective: Chronic obstructive pulmonary disease (COPD) is described as partially reversible airflow limitation. P-glycoprotein (P-gp/MDR1), encoded by the Multidrug Resistance 1 (*MDR1*) gene, is regarded as a protective component for the respiratory tract and is present in tracheobronchial epithelium and lung parenchyma, and removes particles from cells and protects against various xenobiotics. Polymorphisms of *MDR1* gene and the alteration in the expression of P-gp are considered to have a negative effect on the severity of COPD pathogenesis and treatment efficacy. We aimed to investigate the relationship of the *MDR1* gene polymorphisms with reversibility in COPD patients.

Materials and Methods: The *MDR1* polymorphisms, specifically the 3435C>T and 2677A/G variations, were analyzed in 90 COPD patients.

Results: 15 of the 90 COPD patients had positive reversibility tests. 2677TT (p=0.044) and 3435TT (p=0.003) alleles related to positive reversibility tests. There were no significant differences in the distribution of the *MDR1* C3435 alleles and the G2677 alleles (p> 0.05).

Conclusion: COPD patients with the TT allele have a higher rate of early reversibility positivity; this suggests that those carrying the allele may respond better to bronchodilator therapy. These markers could help to distinguish COPD patients who respond better to β2-agonists or who may not benefit much and, therefore, need different drugs.

Keywords: COPD, MDR1, polymorphism

INTRODUCTION

Chronic obstructive pulmonary disease (COPD), which may cause considerable mortality and morbidity, is a significant health issue worldwide.[1] As an external factor, smoking may contribute to the progression of COPD by affecting the detoxification system and causing an imbalance in the protease-antiprotease system.[2] The airway epithelium protects from irritants breathed in and reduces the absorption of foreign substances.

The pulmonary epithelium of the airway is the first barrier for drug delivery following inhalation. The amount of target molecule that reaches the final site of action through the epithelium can be reduced by blood flow, absorption, surface binding, mucociliary clearance, and metabolism.[3] Transporters in the pulmonary epithelium, the first barrier for inhaled drugs, may play a vital role in delivering drugs administered by inhaler. The plasma membrane glycoprotein (P-gp) may limit the absorption of substances breathed in through the bronchial epithelium. P-gp expression occurs in ciliated collecting ducts and epithelial cells or bronchial glands in the human lung.[4] The presence and functions of many ABC transporters are essential for the application of drugs to the site of action, and multidrug resistance-associated protein 1 (MRP1) is amongst ATP binding cassette (ABC) transporters. However, changes in the Multidrug Resistance 1 (*MDR1*) gene's genetic structure or the alteration of P-gp expression may change its functions.[5]

The *MDR1* gene is located in human chromosome 7 and encodes P-gp (170-kDa). This P-gp belongs to the ABC transporters family, also named ABCB1. There are 28 exons (49 to 209 base pairs) in the *MDR1* gene, and it encodes an mRNA (4.5 kb). More than 50 SNPs and insertion/deletion polymorphisms were identified in the *MDR1* gene.[6] Most SNPs are silent (synonymous), and no change can be seen in the amino acid sequence. In the different ethnic populations, 1236C>T and 2677G>T/A/C polymorphisms were detected in the MDR1

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gene. The most commonly seen polymorphism was 3435C>T.[7] Specifically, the C3435T single nucleotide polymorphism identified in exon 26 is considered to be associated with P-gp levels and substrate uptake.[8] Although some studies indicate the role of altered expression of P-gp and the *MDR1* gene polymorphisms for the development of respiratory diseases, their exact role and clinical relevance are not fully understood.

COPD is described as airflow limitation, and a full recovery is impossible. Treatments include bronchodilator drugs for these patients. Variabilities for bronchodilator response (BDR) in COPD patients may be associated with several factors such as age, baseline lung function, and eosinophil biomarkers.[9-11] Depending on these findings, the differences in COPD patients may be related to interindividual variability in the pharmacological response to bronchodilator drugs β2-agonist bronchodilators used for symptomatic treatment in COPD. Genetic variants that determine the bronchodilator response in COPD are being investigated. These markers could help to find COPD patients who respond better to β2-agonists or who may not benefit much and, therefore, need different drugs.

In this study, we aimed to investigate the relationship of the *MDR1* gene polymorphisms with reversibility in COPD patients.

MATERIALS AND METHODS

Study Design

A cross-sectional, real-life prospective study is compatible with the ethical guidelines of the Declaration of Helsinki and was approved by our Institutional Ethics Committee Board (No: 3773 Date: 06.02.2007). Each patient or their relatives gave signed informed consent forms.

Settings

Eligible patients were recruited between January 2010 and July 2010 in our department's COPD outpatient clinics.

Participants

Patients with COPD over 40 years of age who had stopped smoking at least five years ago were included in the study. The patients were in a stable period. Patients who had cancer, cardiac disease, and a COPD attack in the last three months were excluded from the study. Patients with a family history of atopy and allergic complaints with an eosinophil of more than 3% in plasma and patients with positive skin tests for allergies were excluded from the study.

Blood Analysis

Five ml of blood was collected from each patient. Blood samples taken in vacuum sterile K3-EDTA tubes were stored at -20°C, and their DNAs were isolated within the first week. DNA isolation was performed using the Roche DNA kit. The polymorphisms of *MDR1* C3435T and G2677T/As were detected by the PCR-RFLP method.

Pulmonary Function Tests

All subjects performed standardized spirometry according to European Respiratory Society guidelines. It was ensured that the patients did not use bronchodilator drugs for 24 h before the pulmonary function test. A pulmonary function test was performed 15-20 min after Salbutamol 400 mcg, and the response to the bronchodilator was measured by reversibility test. The reversibility test was considered positive if forced expiratory volume (FEV1) increased by 200 mL and the expected FEV1 percentage increased by 12%.

The COPD patients were divided into mild, moderate, and severe according to their FEV1 values. Those with FEV1>80% were assessed as mild, 80%>FEV1>50% as moderate, and those with FEV1<50% as severe. Patient groups were determined according to the GOLD 2005 update.

Study Size

Three groups of 30 patients, each with mild, moderate, and severe obstruction, were included in the study.

Statistical Analysis

All analyses were performed using Epi Info Software version 3.2.2 (CDC, Atlanta, GA). The MDR1 gene polymorphism distribution was compared using χ2 or Fisher test. Significance was concluded with a p-value $\leq$ 0.05. When the p-value was < 0.05, the odds ratio with a 95% confidence interval was calculated.

RESULTS

Participants

150 consecutive COPD patients were studied. Sixty patients were excluded because they did not meet the criteria. Ninety patients with a smoking history of more than 20 packs/year participated in this study. Patients with mild, moderate and severe obstruction were adjusted to 30 people each, and patient recruitment was carried out.

Descriptive and Outcome Data

Subjects (n=90, M/F: 83/7) mean age was 62.3 ± 12.4 years. The smoking duration of the patients was 38.1 ± 19.4 packs/year. The patients' mean FEV1 and FEV1/FVC (Forced vital capacity) values were 1804 ± 444 ml and 55.6 ± 5.5%, respectively (Table 1).

The Polymorphisms of *MDR1* C3435T and G2677T/As

The allele frequencies for the C3435 single nucleotide polymorphism of the *MDR1* gene for COPD patients were detected and recorded (Table 2, Figure 1). C alleles distribution of the*MDR1* gene was found to be 47.7%, and T alleles were found to be 53.3%. CC alleles distribution of the MDR-1 was found (n:22) 33.3%, and CT and TT alleles were detected as (n:42) 51.1% and (n:26) 31.9%, respectively, in COPD patients. The allele frequencies for G2677 single nucleotide polymorphism of the MDR1 genes were determined (Table 3, Figure 2). The *MDR1* gene G allele distribution was 49.4%, the T allele was 87.3%, and the A allele was 0.2% in the COPD group. The MDR genotype distribution was found to be 24.4% for GG, 46.7% for GT, 24.4% for TT, 0.3% for GA, and 0.1% for TA in the COPD group.

Early reversibility test was positive in six patients with mild obstruction, five with moderate obstruction, and four with severe obstruction (a total of 15). No statistical difference was detected between the COPD groups. Significant differences were found between the C3435 polymorphism distribution and the G2677 polymorphism distribution between reversibility positive (n:15) and negative groups (n:75) (Table 4).

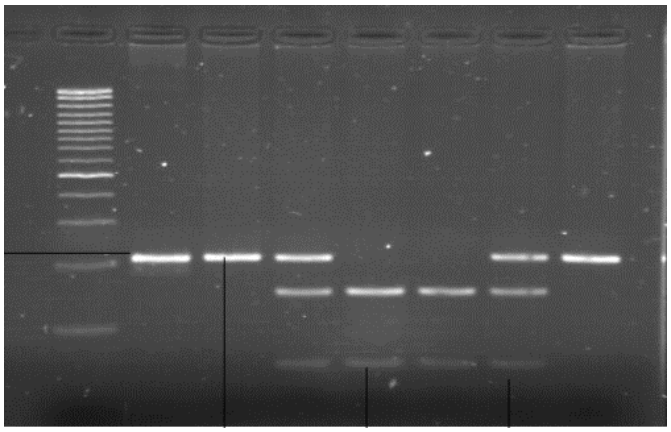

Figure 1. Agarose gel images of *MDR1* polymorphism C3435T genotypes.

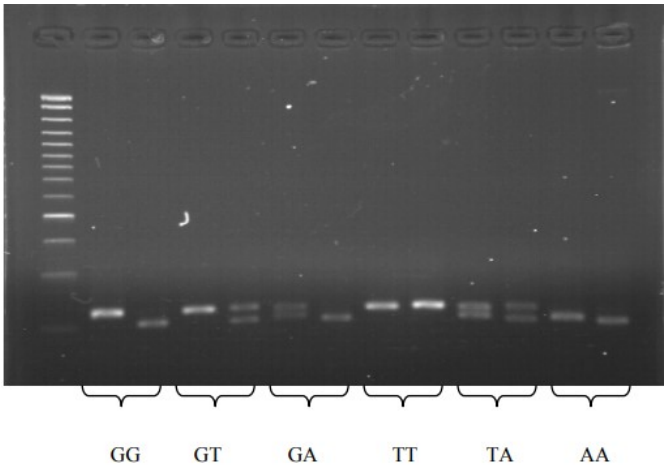

Figure 2. Agarose gel images of *MDR1* polymorphism G2677T genotypes.

DISCUSSION

P-gp protein is vital in decreasing the toxic effect of smoking and removing oxidative stress metabolites.[12] It was found that the bronchial epithelium of COPD patients has been shown to have small quantities of MDR proteins.[13] It was suggested that the *MDR1* gene may interfere with the progression of COPD by detoxification and inflammatory mechanisms. At the site of action, some multidrug resistance proteins may behave like drug efflux pumps, causing a decrease in intracellular concentrations of toxic compounds.[14] These transporters, such as P-glycoprotein and other MDR proteins, are expressed strongly in the respiratory tract. They may help prevent harmful substances from getting into the lungs, whether inside or outside the body.[15]

Other studies have also shown that the SNPs of the MDR1 gene affect how drugs are absorbed, distributed, and eliminated in the body.[16,17] The effects or clinical implications of these polymorphisms on P-gp function are often unknown. However, some of the SNPs have a functional role and can affect how drugs are metabolized in the body. The C3435T, G2677T/A, and C1236T polymorphisms have been studied for exon 26, 21, and 12, respectively, in different populations.[18,19] The importance of the *MDR1* gene polymorphisms and the alteration in the expression level of P-gp protein for respiratory diseases is still not fully understood. Still, researchers suggest that the MDR1 gene polymorphism can be clinically significant for the pathogenesis of COPD.[20] The TT genotype was detected frequently for the C3435 *MDR1* gene in COPD patients, and the *MDR1* gene C/T polymorphism is suggested to have a role in the progression of COPD.[21] We did not find significant differences in the distribution of the *MDR1* gene C3435 alleles and G2677 alleles in our study population. A silent polymorphism (the C3435T SNP) of the *MDR1* gene in exon 26 may cause protein synthesis with the same amino acid sequence but not the same structural and functional properties. Some studies have suggested that certain disease conditions may develop and worsen because of silent SNPs.[22] It is known that airflow obstruction is generally not reversible in COPD. The relationship between many factors and early reversibility in COPD was investigated. It has been shown that the proportion of eosinophils in bronchoalveolar lavage fluid and continued smoking are associated with response to bronchodilator drugs. Hemopoietic cell kinase and β2-adrenergic receptor gene polymorphisms are suggested to be associated with BDR in COPD patients.[23,24] On the other hand, the genetic determinants of BDR in COPD patients are not known. In our study, 15 of 90 COPD patients had positive

Table 1. Clinical parameters in patients with COPD severity. **FEV1:**Forced expiratory volume; **FVC:** Forced vital capacity; **MEFR:**The maximum expiratory flow rate.

	FEV1/FVC %	FEV1 (mL)	FEV1 %	FVC (mL)	FVC %	MEFR (mL)
Mild COPD	65	2590	86	3980	106	1383
Moderate COPD	61	1894	65	3083	86	958
Severe COPD	41	928	32	2246	62	352

Table 2. Distribution of C3435 polymorphism in patient group.

	Mild obstruction n=30	Moderate obstruction n=30	Severe obstruction n=30	p
C/C	8 (26.7 %)	8 (26.7 %)	6 (20 %)	**>0.05**
C/T	14 (46.6 %)	14 (46.6 %)	14 (46.6 %)	
T/T	8 (26.7 %)	8 (26.7 %)	10 (33.3 %)	
C	30 (50 %)	30 (50 %)	26 (43.3 %)	**>0.05**
T	30 (50 %)	30 (50 %)	34 (56.7 %)	

Table 3. Distribution of G2677 polymorphism in patient group.

	Mild obstruction n=30	Moderate obstruction n=30	Severe obstruction n=30	p
G/G	7 (23.3 %)	9 (30 %)	6 (20 %)	**>0.05**
G/T	15 (25 %)	14 (46.7 %)	13 (43.3 %)	
T/T	7 (23.3 %)	6 (20 %)	9 (30 %)	
G/A	0	1 (3.3 %)	2 (6.7 %)	
T/A	1 (1.7 %)	0	0	
G	29 (48.3 %)	33 (55 %)	27 (45 %)	**>0.05**
T	30 (50 %)	26 (43.3 %)	31 (51.7 %)	
A	1 (1.7 %)	1 (1.7 %)	2 (0.3 %)	

Table 4. Distribution of *MDR1* polymorphism in patient group.

Polymorphism	n	*Frequency of Allele*
MDR1 3435		
C/C	4 (26.6 %)	**C allele:** 33.3 %
C/T	2 (13.3 %)	**T allele:** 66.6 **%**
T/T	9 (60 %)	
MDR1 2677		
G/G	2 (13.3 %)	**G allele:** 26.6 %
G/T	3 (20 %)	**T allele:** 66.6 %
T/T	8 (53.3 %)	**A allele:** 6.6 %
G/A	1 (6.6 %)	
T/A	1 (6.6 %)	

reversibility tests. 2677TT (p=0.044) and 3435TT (p=0.003) alleles related to positive reversibility test.

Recent indications in the literature indicate that polymorphisms of the *MDR1* gene play an essential role in the pathogenesis and treatment of respiratory diseases.[13] In addition, in studies conducted in Turkey, it was determined that the TT genotype of the *MDR1* gene was significantly more common in COPD patients.[20,25]

A study showed that there was no significant difference between the genotypes of healthy individuals and the control group consisting of patients with chronic obstructive pulmonary disease and comorbid type 2 diabetes.[26]

The strength of this study is this issue is a point that can guide COPD treatment. A strength of our research is that it raises awareness that specific genetic variations affect people's response to bronchodilators and that bronchodilator sensitivity may differ between different types of COPD. The limitation of this study is a few patients have been included, which is insufficient to draw a clear conclusion about MDR1 genotyping's clinical relevance for treating COPD patients.

CONCLUSION

Our results suggest that bronchodilator responsiveness phenotypes in COPD patients were linked to variations in the *MDR1* C3435 and 2677 genes. Various factors may influence how COPD patients respond to bronchodilators, such as different disease subtypes, how they break down drugs, or other effects related to their genes. The following steps are to repeat this study in diverse populations, identify the specific genetic variations that affect how people respond to bronchodilators, and investigate whether bronchodilator responsiveness varies across different types of COPD. Extensive population studies with more patients are needed to investigate this.

Ethics Committee Approval: This study is compatible with the ethical guidelines of the Declaration of Helsinki and was approved by our Institutional Ethics Committee Board (No: 3773 Date: 06.02.2007)

Informed Consent: Written consent was obtained from the relatives.

Peer Review: Externally peer-reviewed.

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