

## CLINICAL SCIENCE

# Frequency of the *mdr-1* C>T gene polymorphism in patients with COPD

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**BACKGROUND AND AIM:** The multi-drug resistant-1 (MDR-1) gene is located on human chromosome 7 and encodes a glycosylated membrane protein that is a member of the ATP-binding cassette transporters superfamily. The aim of the study was to reveal the role of the C3435T MDR-1 gene polymorphism in chronic obstructive pulmonary disease.

**METHOD:** DNA samples from 41 patients with chronic obstructive pulmonary disease and 50 healthy control participants were used to compare MDR-1 gene profiles. Genotyping assays were performed using the StripAssay technique that is based on reverse-hybridization.

**RESULTS:** The T allele polymorphism in the MDR-1 gene located at position 3435 in exon 26 was shown to correlate with chronic obstructive pulmonary disease.

**CONCLUSION:** These preliminary results suggest that the T allele polymorphism of the MDR-1 gene is associated with chronic obstructive pulmonary disease.

**KEYWORDS:** COPD; MDR-1 gene; T allele frequency; Transporter glycoprotein; Reverse-hybridization.

Dogan OT, Katrancioğlu N, Karahan O, Sanlı GC, Zorlu A, Manduz Ş. Frequency of the *mdr-1* C>T gene polymorphism in patients with COPD. Clinics. 2010;65(11):1115-1117.

Received for publication on June 16, 2010; First review completed on August 17, 2010; Accepted for publication on August 17, 2010

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## INTRODUCTION

Increasing evidence suggests that pulmonary and systemic inflammatory processes play an important role in the development and progression of chronic obstructive pulmonary disease (COPD), which is characterized by partially reversible airway obstruction.<sup>1</sup> Genetic factors, in addition to oxidative stress factors (e.g., smoking), have also been implicated in the development of this disease.<sup>2</sup>

The underlying genetic causes have been evaluated in several studies to clarify the etiopathogenesis of the systemic effects of COPD. The molecular basis of inflammation in COPD has also been investigated.<sup>3</sup> In addition, studies have addressed the protective genetic factors that combat oxidant damage, which is caused by smoke and related agents. It has been suggested that multidrug resistance (MDR) proteins may protect lung tissue from oxidative stress by acting as anti-oxidants.<sup>4</sup> MDR genes constitute a class of genes that play a crucial role in multiple drug resistance in eukaryotic cells. The MDR-1 gene

encodes P-glycoprotein (P-gp), which plays a role in the active transport of various substrates. More specifically, P-gp plays an important role in the excretion of toxic substances, ingestion of nutrients, transport of ions and peptides, and transduction of cellular signals. Notably, it is an adenosine triphosphate (ATP)-dependent transport protein. P-gp carries free cholesterol from the plasma membrane to the endoplasmic reticulum, where cholesterol esterification occurs.<sup>5-7</sup> The single nucleotide polymorphism (SNP) C3435T located in exon 26 of the MDR-1 gene results in decreased expression of P-gp.

In the present study, we aimed to evaluate the relationship between COPD and the MDR-1 gene, which has been postulated to play a role in the inflammatory process and protection against oxidative stress.

## MATERIALS AND METHODS

Ninety-one participants were selected for this study. Of these, forty-one were diagnosed with COPD (i.e., emphysema) and comprised the study group. The remaining fifty participants were healthy control participants. The clinical diagnosis of COPD was made by a computed tomography (CT) scan. Participants with additional systemic disorders were excluded from the study. After informed consent was obtained, a 5-ml peripheral blood sample was taken from each participant for genetic analysis.

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### Polymorphism Analysis:

Total genomic DNA was extracted from 100- $\mu$ l blood samples using the Invitex kit (Invitex, Invisorb spin blood, Germany). The MDR-1 gene was amplified in a biotin-labeled single multiplex amplification reaction and evaluated for the 3435 C>T polymorphism. **Polymerase chain reaction (PCR)** was performed in a Perkin Elmer GeneAmp 9600 Thermal Cycler. The protocol consisted of an initial melting step of 2 minutes at 94°C, followed by 35 cycles of 15 seconds at 94°C, 30 seconds at 58°C and 30 seconds at 72°C, and a final elongation step of 3 minutes at 72°C. Polymorphism analysis was performed using the Strip Assay technique (Vienna Lab, PGX-HIV Strip Assay GmbH, Austria), which is based on reverse-hybridization.

Data from the study population and the healthy control group were statistically analyzed using the SPSS software program (SPSS Inc. Chicago, IL, USA).  $P < 0.05$  was considered to indicate statistical significance.

## RESULTS

The study group was composed of 24 males (59%) and 17 females (41%); the control group was composed of 29 males (58%) and 21 females (42%). The mean age of the study group was  $62.3 \pm 7.3$  years and that of the healthy control participants was  $57.8 \pm 12.4$  years. Thirty-five (85%) and thirty-one (62%) of the study and the control participants, respectively, had a history of smoking.

MDR-1 CC (wild type), CT (heterozygous) and TT (homozygous) gene polymorphisms were compared between the study and control groups. The wild type, heterozygous, and homozygous gene polymorphisms were found in 9 (22%), 21 (51%), and 11 (27%) of the study participants, respectively. In the control group, the wild type, heterozygous, and homozygous gene polymorphisms were found in 35 (70%), 11 (22%), and 4 (8%) participants, respectively (Table 1).

This comparison showed that both MDR-1 homozygous and heterozygous polymorphisms were significantly more frequent in patients with COPD ( $p < 0.05$ ).

## DISCUSSION

COPD can lead to death and is the fourth most frequent cause of death in Europe and the United States.<sup>8</sup> It is important to clearly elucidate the etiopathogenesis of this disease to prevent death in patients and to develop new therapeutic modalities. There is increasing evidence that indicates that the inflammatory process in COPD patients is activated (e.g., an increase in circulatory cytokines, chemo-

kines, and acute phase reactants, particularly during exacerbation and progression of the disease).<sup>1,9,10</sup>

Smoking, in addition to genetic and other environmental factors, contributes to the development of COPD. Smoking overworks the detoxification system by causing an imbalance within the protease-anti-protease system. Various studies have assessed the molecular mechanisms of these systems.<sup>11</sup> The MDR-1 gene, which is located on chromosome 7, encodes P-gp, a transmembrane efflux pump that transports drugs and toxins from the intracellular to the extracellular domain. The C3435T single nucleotide polymorphism located in exon 26 of the MDR1 gene was recently shown to be associated with P-gp levels and substrate uptake. Individuals who were homozygous for the T-allele had a significantly decreased P-gp expression level compared to those homozygous for the C-allele.

Previous literature has shown that the MDR-1 3435 C>T polymorphism may be important in Parkinson disease, inflammatory bowel diseases, cerebral artery aneurisms, refractory epilepsy, and **cluster of differentiation 4** (CD4) cell regeneration during HIV (Human Immunodeficiency Virus) infection. It has also been shown to influence drug levels in the body.<sup>7,12,13</sup> In other studies, it was suggested that P-gp protein plays a role in combating the toxic effects of smoking and in the removal of oxidative stress metabolites.<sup>14,15</sup> The gene has also been shown to play a role in cellular regeneration.<sup>16</sup> In a study by van der Deen et al., MDR proteins were shown to exhibit a protective effect against oxidative stress and that COPD patients have decreased levels of Multidrug resistance-associated protein-1 (MRP-1) in their bronchial epithelium.<sup>4</sup> It has also been established that pro-inflammatory cytokines decrease the amount of products (e.g. MRP-1) secreted from the cell by P-gp.<sup>17</sup> In other studies, a decrease in P-gp expression and activity was observed during inflammation.<sup>18</sup> Similarly, decreased expression of P-gp has been shown in individuals with the MDR1 polymorphism.<sup>12</sup> In the present study, we investigated the MDR-1 3435 C>T polymorphism in COPD patients. In the study group, the wild type, heterozygous, and homozygous MDR1 gene polymorphisms were found in 9 (22%), 21 (51%), and 11 (27%) of the participants, respectively, whereas in the control group, they were found in 35 (70%), 11 (22%), and 4 (8%) of the participants. These results indicate a distribution of the C/C, T/T, and C/T genotypes in patients with COPD of 22%, 51%, and 27%, respectively. The T-allele frequency was significantly higher ( $P < 0.05$ ) in COPD patients when compared to the healthy control participants (52% and 19%, respectively), (Tables I and II).

This pilot study had some limitations due to its retrospective nature. Specifically, the phenotypic reflection of the

**Table 1** - Distribution of MDR-1 (P-glycoprotein, ATP-binding cassette ABC superfamily of membrane transporters) genotypes in patients with COPD and healthy control participants.

Group	Genotype			Total
	CC (Wild Type)	CT (Heterozygous)	TT (Homozygous)	
COPD n (%)	9 (22%)	21 (51%)	11 (27%)	41 (100%)
Control n (%)	35 (70%)	11 (22%)	4 (8%)	50 (100%)
p*	0.000	0.004	0.016	

\* $p < 0.05$

**Table 2** - Allelic frequency of the MDR-1 gene (P-glycoprotein, ATP-binding cassette ABC superfamily of membrane transporters) C3435T polymorphism in patients with COPD and healthy control participants.

Group	Allele		Total
	C	T	
COPD n (%)	39 (48%)	43 (52%)	82 (100%)
Control n (%)	81 (81%)	19 (19%)	100 (100%)
p*	0.000	0.000	

\* $p < 0.05$

resulting genotype distribution was not assessed. In addition, neither P-gp nor MRP-1 levels were evaluated. However, the primary purpose of this study was to obtain a genetic reference for future studies by assessing a limited population. Thus, our investigation focused on evaluating the different MDR-1 gene polymorphisms.

Our results show that the CT and TT MDR-1 gene polymorphisms were significantly more frequent in COPD patients. These findings suggest that MDR-1 may play a role in the development of COPD through some inflammatory and detoxification mechanisms.

More detailed studies focusing on phenotypic alterations are necessary to clarify our findings. In addition, elucidating the molecular etiopathogenesis of COPD should open new frontiers in identifying populations at risk and in developing new treatment and follow-up modalities.

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