Int J Clin Exp Med 2014;7(10):3573-3577
www.ijcem.com /ISSN:1940-5901/IJCEM0001895

Original Article

MDR-1 gene C/T polymorphism in COPD: data from Aegean part of Turkey

Ümran Toru[1], Ceylan Ayada[2], Osman Genç[2], Sebahat Turgut[3], Günfer Turgut[3], İsmet Bulut[4]

[1]Department of Chest Diseases, Faculty of Medicine, Dumlupınar University, Kütahya 43100, Turkey; [2]Department of Physiology, Faculty of Medicine, Dumlupınar University, Kütahya 43100, Turkey; [3]Department of Physiology, Faculty of Medicine, Pamukkale University, Denizli 20070, Turkey; [4]Department of Allergic Disease, Süreyyapaşa Chest Diseases and Thoracic Surgery Training and Research Hospital, Istanbul 34844, Turkey

Received August 14, 2014; Accepted September 20, 2014; Epub October 15, 2014; Published October 30, 2014

Abstract: Objective: Genetic factors, in addition to oxidative stress factors, have been implicated in the development of chronic obstructive pulmonary disease (COPD). Multi-drug resistant-1 (MDR-1) is a gene located on chromosome 7 and the products of this gene protect lung tissue from oxidative stress. We searched the frequency of MDR-1 gene C/T polymorphism in patients with COPD and aimed to explain the association between MDR-1 gene and COPD development. Methods: 47 patients with COPD and 64 healthy control participants were placed in this study. DNAs were extracted from blood samples and MDR-1 amplification of DNA was performed using polymerase chain reaction and enzyme digestion techniques. Results: The frequencies of MDR-1 genotypes were found 17.0% for CC, 51.1% for CT and 31.9% for TT in the COPD group and 39.1% for CC, 53.1% for CT and 7.8% for TT in the control group. The distribution of MDR-1 gene C alleles were found 32.3% in COPD group and 67.7% in control group; T alleles were found 55.1% in COPD group and 44.9% in control group. There was statistically significant difference between the groups for genotype and allele frequency of MDR-1 gene (P = 0.001). Conclusion: TT genotype of MDR-1 gene was significantly more frequent in COPD patients. MDR-1 gene C/T polymorphism may play a role in COPD development.

Keywords: Multi-drug resistant-1 gene, polymorphism, chronic obstructive pulmonary disease

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by an excessive and chronic inflammatory response of the lungs to respiratory pollutants, mainly tobacco smoking [1]. Chronic inflammation of the peripheral airways and lung parenchyma leads to partially reversible but progressive obstruction of the airways [2]. The molecular basis of inflammation in COPD has been investigated and the underlying genetic causes of this disease have been evaluated in several studies [3].

It was shown that both pulmonary and systemic inflammatory processes play an important role in the development and progression of COPD [4]. Also genetic factors, in addition to oxidative stress factors (e.g., smoking), have also been implicated in COPD development [5].

Multi-drug resistant-1 (MDR-1) is a gene located on chromosome 7q21 [6]. The products of this gene, such as multidrug resistance-associated protein-1 (MRP1), permeability-glycoprotein (P-gp) and lung resistance-related protein (LRP), act as anti-oxidants and protect lung tissue against oxidative stress and toxic compounds generated by cigarette smoking [7]. MDR-1 encodes P-gp, which is a transmembrane efflux pump that transports drugs and toxins from intracellular to extracellular area [8]. Studies have suggested that proteins produced by MDR-1 gene protect lung tissue from oxidative stress by acting as anti-oxidants [7].

In this study, we aimed to investigate genotype and allele frequencies of MDR-1 gene C/T polymorphism in patients with COPD in Kütahya province of Turkey. Additionally, we examined to explain the relationship between MDR-1 gene and COPD development.

Table 1. Hardy-Weinberg equilibrium of the C/T polymorphism in MDR gene

		COPD		Control	
	Genotype	Expected	Observed	Expected	Observed
Common homozygotes	CC	8.51	8	27.56	25
Heterozygotes	CT	22.98	24	28.88	34
Rare homozygotes	TT	15.51	15	7.56	5
		$X^2 = 0.09$, $p > 0.05$		$X^2 = 2.02$, $p > 0.05$	

Methods

Participants

47 COPD patients who are on-clinical follow-up at Dumlupınar University, Department of Chest Diseases, Kütahya (a city located in the Aegean part of Turkey) and 64 healthy age-matched subjects were involved in our study. The diagnosis of COPD was established on the basis of criteria proposed by Global Initiative for Chronic Obstructive Lung Disease (GOLD). Both control and patient groups were chosen among Turkish population and they were ethnically Caucasian. Individuals who have comorbidities were excluded from the study. All of the procedures were explained to the subjects and written informed consent was obtained from all individuals. The study protocol conforms to the ethical guidelines of Declaration of Helsinki and was approved by the Ethics Committee of Afyon Kocatepe University.

Genotyping

DNA isolation: Blood samples of 111 participants (47 COPD patient, 64 heathy control) were collected in tubes with EDTA. DNA samples were isolated from peripheral blood leukocytes by standard phenol/chloroform extraction method.

Polymerase chain reaction: Polymerase chain reaction (PCR) was used to detect C3435T single nucleotide polymorphism (SNP). A PCR assay using the forward primer MDR1F 5'-TGC TGG TCC TGA AGT TGA TCT GTG AAC-3' and the reverse primer MDR1R 5'-ACA TTA GGC AGT GAC TCG ATG AAG GCA-3' was performed with 10× buffer, 1.5 mM $MgCl_2$ and 0.2 mM each dNTP and 1 U Taq DNA polymerase [9].

PCR amplification consisted of an initial denaturation for 2 min at 94°C followed by 35 cycles of denaturation at 94°C for 30 s, annealing temperature 60°C for 30 s, and extension at 72°C for 30 s. Terminal elongation was performed at 72°C for 4 min. The digestion of a 248-bp PCR product with restriction enzyme MboI for 2 h at 37°C followed this step. Digested products were separated on a 3% agarose gel with ethidium bromide. Subsequently, restriction fragments were identified using the UVI Gel Documentation system. Fragments obtained were 238 bp to T/T genotype, 172 bp and 60 bp fragments to the C/C genotype, and 238 bp, 172 bp and 60 bp to the C/T genotype [10].

Statistical analysis

Statistical analyses were performed by SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) 16.0 package program. All data were given as mean ± standard deviation (SD). Chi-square test was done for the comparison of nominal variables between the groups. Hardy-Weinberg equilibrium was evaluated by the chi-square test. Statistical significance of the observed genotype frequencies was evaluated according to Hardy-Weinberg rule by comparison of the expected genotype frequencies. *P* values < 0.05 were considered as statistically significant.

Results

The frequency of MDR genotype C/T polymorphism in control and patient groups did not show a significant deviation from Hardy-Weinberg equilibrium ($P > 0.05$) (**Table 1**). The frequencies of MDR genotype in COPD patients and control subjects were shown in **Table 2**. The distribution of MDR genotypes were found (8) 17.0% for CC, (24) 51.1% for CT, and (15) 31.9% for TT in COPD group and (25) 39.1% for CC, (34) 53.1% for CT, and (5) 7.8% for TT in control group.

Statistically significant difference was observed between the groups for MDR genotypes ($X^2 = 13.544$; df = 2; $P = 0.001$).

The allele frequencies for MDR-1 gene in COPD patients and control subjects were shown in **Table 2**. The distribution of MDR-1 gene C alleles were found (40) 32.3% in COPD group

Table 2. Genotype and allele frequencies of the C/T polymorphism in MDR gene

	COPD		Control	
	n	%	n	%
Genotype Frequency				
MDR C/T polymorphism				
CC	8	17.0	25	39.1
CT	24	51.1	34	53.1
TT	15	31.9	5	7.8
Total	47		64	
X^2 = 13.554; df = 2; P = 0.001				
Allele Frequency				
MDR C allele	40	32.3	84	67.7
MDR T allele	54	55.1	44	44.9
X^2 = 10.784; df = 1; P = 0.001				

and (84) 67.7% in control group; T alleles were found (54) 55.1% in COPD group and (44) 44.9% in control group. There was statistically significant difference between the groups for allele frequency (X^2 = 10.784; df = 1; *P* = 0.001) (**Table 2**).

Discussion

It is a known fact that tobacco smoking is the principal risk factor for COPD development [7]. Smoking overworks the detoxification system by causing an imbalance within the protease-anti-protease system [11]. Although COPD is mainly associated with cigarette smoking, only a small portion-approximately 20%-of smokers develop this disease. Because there are additional factors such as environmental, genetic and epigenetic components, which take part in the pathogenesis of this disease [12-14]. Combined influence of these risk factors alters lung development and immunity so this leads to the predisposition for COPD [15]. Although environmental factors have a direct role in the pathogenesis of COPD, genetic risk factors also appear to play a fundamental role in disease susceptibility and severity [1]. Unfortunately the molecular and cellular mechanisms that lead to COPD development are poorly understood [15].

The C3435T SNP located in exon 26 of the MDR-1 gene is a silent polymorphism that can lead to the synthesis of protein product with the same amino acid sequence but different structural and functional properties [16, 17]. It was reported that silent SNPs may contribute to development and progression of certain disease conditions [18-20]. Also, this SNP of MDR-1 gene was shown to be associated with P-gp levels [8].

P-gp, also called as multiple drug resistance protein, plays a role in combating the toxic effects of smoking and in the removal of oxidative stress metabolites [21-23]. Piquette-Miller et al., observed a decrease in P-gp expression and activity during inflammation [24]. Also, decreased expression of P-gp has been shown in individuals with MDR-1 polymorphism [16]. Hoffmeyer et al. reported that 3435C > T was associated with significantly reduced intestinal P-gp expression in T/T homozygotes in comparison with subjects homozygous for C allele (C/C) [25]. In a study by Gümüş-Akay et al., decreased expression of P-gp in individuals who were homozygous for the T-allele of MDR-1 gene was observed compared to those homozygous for the C-allele [16]. Dogan et al., showed that both MDR-1 homozygous (TT) and heterozygous (CT) polymorphisms were significantly more frequent in patients with COPD [8]. However, we could not evaluate plasma P-gp levels of the individuals. This was a limitation of our study.

In our study, we found a statistically significant difference between the groups for genotype and allele frequency of MDR-1 gene. We detected that TT genotype of MDR-1 gene were significantly more frequent in COPD patients.

According to our results, we suggest that increased T-allele frequency of MDR-1 gene in these patients may lead more exposure to oxidative stress which can result in COPD development. By this way, MDR-1 C/T polymorphism may contribute to genetic susceptibility of COPD.

Conclusion

We can conclude that MDR-1 gene C/T polymorphism may play a role in COPD development. Identifying the genetic background of patients with COPD provide us to determine risk groups in the community and develop new individual-based targets for therapy. Furthermore, new studies with large patient populations are required to support our results.

Acknowledgements

This study was supported by Dumlupınar University Scientific Research Fund Commission (Project no: 2013/15).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ümran Toru, Department of Chest Diseases, Faculty of Medicine, Dumlupınar University, Kütahya 43100, Turkey. E-mail: umran_toru_81@hotmail.com

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