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The effect of CYP1A2 gene polymorphisms on Theophylline metabolism and chronic obstructive pulmonary disease in Turkish patients

Ahmet Uslu[1], Candan Ogus[2,], Tulay Ozdemir[2], Turker Bilgen[3], Ozgur Tosun[4] & Ibrahim Keser[3]*

[1]Department of Chest Diseases, Medical Park Hospital, [2]Department of Chest Diseases, Faculty of Medicine, Akdeniz University, [3]Department of Medical Biology and Genetics, Faculty of Medicine, Akdeniz University, [4]Department of Biostatistics and Medical Informatics, Faculty of Medicine, Akdeniz University, Antalya, Turkey

Cytochrome P450 (CYP) 1A2 gene polymorphisms are thought to be involved in the metabolism of theophylline (TP). We aimed to investigate the effect of *CYP1A2*1C, CYP1A2*1D, CYP1A2*1E,* and *CYP1A2*1F* polymorphisms of the *CYP1A2* on TP metabolism by PCR-RFLP in 100 Turkish patients with chronic obstructive pulmonary disease (COPD) receiving TP. One hundred and one healthy volunteers were included as control group. The genotype frequencies of the *CYP1A2*1D* and *CYP1A2*1F* were found to be significantly different in the patients compared to the controls. The "T" allele at -2467 delT and the "C" allele at -163 C > A in the *CYP1A2* displayed association with a significantly increased risk for COPD. "T" allele at -2467 delT was also associated with a high risk of disease severity in COPD. In conclusion, our data suggest that genetic alterations in *CYP1A2* may play a role both in the pharmacogenetics of TP and in the development of COPD. [BMB reports 2010; 43(8): 530-534]

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease with some significant extra-pulmonary effects. Its pulmonary component is characterized by airflow limitation that is not fully reversible. Bronchodilator medications are the mainstay of symptomatic management of COPD. Combination or individually usage of β_2 agonist, and anticholinergic, and/or theophylline (TP) may produce additional improvements in lung function and health status. TP, the most commonly used methylxanthine, is metabolized by Cytochrome (CYP) P450 mixed function oxidases (1, 2). Among CYP enzymes, *CYP1A2* is prominently involved in biotransformation of TP (3, 4). Due to presence of inter-individual differences in *CYP1A2* activity (5) and the narrow therapeutic window of TP, plasma TP level is monitored currently for optimization and individualization of therapy. Some adverse effects of TP may occur even in therapeutic range. The severity of toxicity may be notably increased by overdose, often with plasma TP levels above 20 μg/ml (4). The most prominent side effects of TP therapy are headache, nausea and vomiting, abdominal discomfort, restlessness, gastro esophageal reflux, and diuresis. Convulsions, cardiac arrhythmias and death may also occur at high concentrations (6).

Both environmental and genetic factors have been described to elucidate the mechanisms underlying the inter-individual differences in *CYP1A2* activity. Among the different *CYP1A2* gene variants defined so far, some have been reported to be responsible for the altered activity of the *CYP1A2* gene. While *CYP1A2*1F* has been associated with increased inducibility, *CYP1A2*1C, CYP1A2*1K, CYP1A2*3, CYP1A2*4, CYP1A2*6, CYP1A2*7, CYP1A2*8, CYP1A2*11, CYP1A2*15*, and *CYP1A2*16* variants have been associated with diminished activity of the *CYP1A2* gene (7-12).

On the other hand, since environmental toxicants are reported among the risk factors for COPD, studies focused on the genetic factors modifying detoxification capacity to describe genetic predisposition. Due to the complex etiological nature of COPD, presence of lots of genetic variations in genes which are generally involved in detoxification mechanism such as *CYP2E1* and *NAT2* have been described as risk factors for COPD (13). *CYP1A2* gene has also been described as a risk factor for COPD in Tatar population but not in Russian population in the same study (14). There is a limited number of reports on the effect of *CYP1A2* on TP metabolism and predisposition to disease in COPD patients. Therefore, we aimed to investigate four known polymorphisms, namely *CYP1A2*1C* and *CYP1A2*1D* in the 5′-flanking region and *CYP1A2*1E* and *CYP1A2*1F* in the first intron of the *CYP1A2* gene in Turkish patients with COPD.

*Corresponding author. Tel: 902422496696; Fax: 902422496040; E-mail: candanogus@akdeniz.edu.tr

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RESULTS AND DISCUSSION

One hundred Turkish patients (81 males and 19 females) with COPD under TP treatment, with a mean age of 64.6 ± 8.4 years, were included in this study. One-hundred-one healthy subjects (33 males and 68 females), whose ages ranged from 40 to 81 (mean age 60.1 ± 7.9 years) and who were not receiving medication were studied as the healthy control group. Forty-three patients with COPD were current smokers, 45 were ex-smokers, and 12 were non-smokers. According to GOLD criteria, severity of COPD was moderate in 23 patients, severe in 51 patients, and more severe in 26 patients.

The distribution of each genotype and allele frequencies in patients with COPD and controls are summarized in Table 1. All genotypes conformed to Hardy-Weinberg equilibrium in the study and control groups. The "T" allele at position -2467 delT and the "C" allele at position -163 C>A of the *CYP1A2* gene showed significantly increased risk for COPD. The "T" allele at position -2467 delT was associated with a high risk of disease severity in COPD (P = 0,021, Chi Square test). The comparisons of severity of COPD according to each polymorphic site are shown in Table 2.

Our results displayed a significant association only between the "T" allele at position -2467 and plasma TP levels. Plasma TP levels adjusted by daily TP dose in patients carrying the "T" allele at -2467 position were significantly low compared to those with the other genotype (P = 0,039, Mann-Whitney U test). Plasma TP levels were 0,0185 ± 0,0023 mg/ml in smokers, 0,0173 ± 0,0013 mg/ml in ex-smokers, and 0,0224 ± 0,0034 mg/ml in non-smokers. There was no association between plasma TP levels and smoking status of the patients (P = 0,375, Kruskal-Wallis test). Plasma TP levels in each genotype are shown in Table 3.

We investigated the pharmacogenetic importance and causative role of *CYP1A2*1C, CYP1A2*1D, CYP1A2*1E*, and *CYP1A2*1F* variants of the *CYP1A2* gene in Turkish COPD patients receiving TP. The role of *CYP1A2* in TP metabolism is well known today and pharmacogenomic importance of genomic alterations in *CYP1A2* are being currently investigated to understand inter-individual variations. Despite plenty of single nucleotide polymorphisms (SNPs) found in the regulatory sequence of *CYP1A2*, some of which have been associated with variation in enzymatic activity, Jiang et al. have reported no SNPs or haplotypes in the *CYP1A2* gene to be associated with the metabolic phenotype in their study (15). However, none of the SNPs we selected for this study has been tested by the authors. Even though there is no report about the functional role of *CYP1A2*1D*, we found that plasma TP levels in patients carrying the "T" allele were lower compared to the TP levels of the patients with delT/delT genotype (P = 0,039, Mann-Whitney U test). This may mean that presence of the "T" allele may be associated with an increase in the *CYP1A2* activity. Although functional roles have been reported for *CYP1A2*1C* and *CYP1A2*1F* (16) variants, no statistically significant difference in blood TP concentrations were found.

Table 1. The distribution of each genotype and allele in patients with COPD (n = 100) and controls (n = 101)

Polymorphism	Genotypes (%)			Allele frequency
*CYP1A2*1C*	G/G	G/A	A/A	
COPD patients (n = 100)	93%	7%	0%	G = 0.965 A = 0.035
Healthy controls (n = 101)	92%	8%	0%	G = 0.96 A = 0.04
*CYP1A2*1D*[a]	T/T	T/del	Del/del	
COPD patients (n = 100)	2%	34%	64%	T = 0.19 del = 0.81
Healthy controls (n = 101)	0%	16%	84%	T = 0.08 del = 0.92
*CYP1A2*1E*	T/T	T/G	G/G	
COPD patients (n = 100)	92%	8%	0%	T = 0.96 G = 0.04
Healthy controls (n = 101)	98%	2%	0%	T = 0.99 G = 0.01
*CYP1A2*1F*[a]	C/C	C/A	A/A	
COPD patients (n = 100)	11%	58%	31%	C = 0.4 A = 0.6
Healthy controls (n = 101)	6,9%	39,6%	53,5%	C = 0.268 A = 0.732

[a]P<0.05 (Chi-square test)

Table 2. The comparison of severity of COPD according to each polymorphic site

Polymorphism		Severity of COPD		
		Moderate (n)	Severe (n)	More severe (n)
*CYP1A2*1C*	G/G	23	45	25
	G/A	0	6	1
*CYP1A2*1D*	T/T + T/del[a]	3	20	13
	del/del	20	31	13
*CYP1A2*1E*	T/T	22	48	22
	T/G	1	3	4
*CYP1A2*1F*	C/C	1	7	3
	C/A	15	29	14
	A/A	7	15	9

[a]P = 0.021 (Chi-Square Test)

Table 3. Association between plasma Theophylline levels adjusted by daily TP dose and polymorphisms

Polymorphisms		Blood theophylline levels (mean ± SD) (mg/ml/daily dose)
*CYP1A2*1C*	G/G	0.0181 ± 0.0095
	G/A	0.0226 ± 0.0064
*CYP1A2*1D*[a]	T/del + T/T	0.0151 ± 0.0100
	del/del	0.0201 ± 0.0087
*CYP1A2*1E*	T/T	0.0186 ± 0.0095
	T/G	0.0171 ± 0.0086
*CYP1A2*1F*	C/C	0.0201 ± 0.0042
	C/A	0.0182 ± 0.0097
	A/A	0.0219 ± 0.0108

[a]P = 0,039 (Mann-Whitney U test)

Additionally, even though it has been reported before, the smoking status had no effect on blood TP concentrations associated with neither *CYP1A2*1D* nor the other variants included in our study. While the decreased activity related to *CYP1A2* may lead to toxic TP levels, increased inducibility may lead to insufficient therapeutic efficiency. Therefore, characterization of *CYP1A2*'s effect on TP metabolism would be meaningful for COPD patients receiving TP for medication, especially for the elderly who have several risk factors that may increase the plasma TP level. As *CYP1A2* is a TP metabolizing enzyme, its pharmacogenomic importance has been studied in Japanese patients receiving oral TP, the vast majority of whom were asthmatic. No significant association has been found between *CYP1A2* enzymatic activity and *CYP1A2* variants (*CYP1A2*1C* and *CYP1A2*1F*) in the mentioned study (17). Contrarily, *CYP1A2*1C* has been associated with altered TP metabolism in asthmatic Japanese patients in another study (18).

In our study, the COPD patients had a higher prevalence of the *CYP1A2*1D* "T" allele and the *CYP1A2*1F* "C" allele compared to the control group. Therefore, these allelic variants may be described as high risk factors for COPD. In addition, the *CYP1A2*1D* "T" allele was found to be associated with the severity level of the disease. The association between the *CYP1A2*1D* "T" allele and disease severity could also support the data describing the T allele as a risk factor for COPD. *CYP1A2*1D* has been described as a risk factor for COPD in Tatar population before (15). Allelic variants of CYP2E1 and NAT2 have been described as risk factors for COPD (13). Due to the discovery of an increasing number of functional SNPs and existence of the common haplotypes in *CYP1A2*, it seems that haplotyping for *CYP1A2* could be more efficient than genotyping for a single polymorphism to evaluate the role of this gene. Sharke *et al*. and Soyama *et al*. have described the haplotypic profiles for Caucasian and Japanese populations, respectively (19, 20). In addition, Soyama *et al*. have reported that haplotype analysis could be more relevant than genotyping a SNP for association studies on pharmacokinetic parameters.

In addition to solitary effects of SNPs included in our study, we evaluated our data to assess the combined effect of *CYP1A2*1D* and *CYP1A2*1F*. We found that individuals carrying both the "T" allele for -2467 delT and the "C" allele for -163 C > A have a higher risk for disease development compared to individuals carrying only one of these variants (P = 0,038, Chi Square Test). Additionally, we found lower plasma TP levels in the patients having both the -2467 delT "T" allele and the -163 C > A "C" allele (0,0148 ± 0,0026 mg /ml), compared to the other patients (0,0209 ± 0,0014 mg /ml). This suggests that combined effect of *CYP1A2*1D* and *CYP1A2*1F* variants on plasma TP levels is more powerful than the solitary effect of each variant (P = 0,038).

Differences in xenobiotic metabolizing capability of the lung might be a determining factor for inter-individual variety in the development of the lung diseases such as COPD. In this sense, high or low activity of CYPs due to genetic variations and/or environmental interactions may contribute to COPD. Bernauer *et al*. have shown that *CYP1A2* is expressed in lung. This links the *CYP1A2* gene to both overexposure to toxicants and to elimination of TP in lung, making it more important for development of COPD and for the patients using TP (21). It would be speculated that local elimination of TP in lung which could be modified by the variation in the CYP1A2 activity could reduce the therapeutic efficiency of TP in target cells, because TP shows its relaxation effect, which is the main therapeutic benefit of TP, directly on airway smooth muscle cells. Thus *CYP1A2* could be described as a factor affecting blood TP levels and also local elimination in the lung (systemic and local pharmacokinetics of the TP). Nevertheless, it is very difficult to know how cellular pharmacokinetics of TP is affected by genetic variations and how *CYP1A2* modify local elimination of TP. It should be kept in mind that *CYP1A2* may alter the therapeutic efficiency of the drug in target cells. Despite systemic pharmacokinetic effect of *CYP1A2* on TP that could be monitored by measuring blood drug concentration, it is not easy to determine the local pharmacokinetic effect of *CYP1A2* on TP. It could be evaluated by further examination assessing the regression in symptoms which are supposed to be provided by TP. In our study, we had only TP blood concentrations allowing us to discuss the potential effect of *CYP1A2* on systemic pharmacokinetics of TP.

TP is not only eliminated by CYP1A2, but it may also be

Table 4. Location of the polymorphisms, primers and the length of PCR products, and Restriction Endonucleases used in PCR-RFLP

Polymorphisms in *CYP1A2*	Primers	Length of PCR products (bp)	Restriction Endonuclease
-3860 G > A *CYP1A2*1C*	F: 5′- GCT ACA CAT GAT CGA GCT ATA C -3′ R: 5′- CAG GTC TCT TCA CTG TAA TGT TA -3′	598	Dde I
-2467 delT *CYP1A2*1D*	F: 5′- TGA GCC ATG ATT GTG GCA TA -3′ R: 5′- AGG AGT CTT TAA TAT GGA CCC AG -3′	167	Nde I
-739 T > G *CYP1A2*1E*	F: 5′- AAA GAC GGG GAG CCT GGG CTA GGT GTA GGA G -3′ R: 5′- AGC CAG GGC CAG GGC TGC CCT TGT GCT AAG -3′	169	Stu I
-163 C > A *CYP1A2*1F*	F: 5′- CCC AGA AGT GGA AAC TGA GA -3′ R: 5′- GGG TTG AGA TGG AGA CAT TC -3′	243	Apa I

metabolized by other CYP enzymes such as CYP2A13, CYP1A1, CYP2E1, CYP2D6, CYP3A4 (21-23). Less is known about these genes' contribution to TP biotransformation. These genes should be considered in further comprehensive studies. Patients who have extremely low or high TP plasma levels should be screened for other known functional and/or possibly functional novel *CYP1A2* variants. We described that the *CYP1A2*1D* variant is associated with the development of COPD, disease severity, altered plasma TP levels, and that *CYP1A2*1F* is related with the risk for development of COPD. Finally, our data acquired from the patients with COPD receiving TP show that genetic alterations in *CYP1A2* gene may play a role both in pharmacogenetics of TP and development of COPD.

MATERIALS AND METHODS

Subjects

Unrelated Turkish patients with COPD receiving TP therapy, who regularly visited the Akdeniz University Hospital, Department of the Chest Diseases, Antalya, Turkey, were included in this study. The last measured plasma TP levels of the patients who had been continuously taking a slow releasing formulation of TP for at least 12 weeks were considered. Patients were diagnosed and classified according to criteria of Global Initiative for Chronic Obstructive Lung Disease (1). Another group of healthy subjects, who were not receiving medication, were studied as age-matched healthy control group. Mild stage COPD patients, who did not receive TP treatment, were excluded from the study. Patients who had had acute COPD attacks within the previous 4 weeks were excluded from the study. Patients with extreme obesity, with renal or hepatic dysfunction, with co-morbidities that could influence TP metabolism such as thyroid dysfunction and cardiac failure, with respiratory failure requiring oxygen therapy, and patients who had taken a drug within the last one week that could affect TP metabolism were excluded from this study. The ethics committee of Faculty of Medicine in Akdeniz University approved this study protocol and signed written consents were taken from all subjects.

CYP1A2 genotyping: *DNA Extraction*: Genomic DNA was isolated from peripheral leukocytes of each subject by a DNA isolation Kit (Molzyme, DE).

PCR-RFLP: -3860 G > A (*CYP1A2*1C*), -2467 delT (*CYP1A2*1D*), -739 T > G (*CYP1A2*1E*), and -163 C > A (*CYP1A2*1F*) were detected by PCR-RFLP using primer pairs and restriction endonucleases shown in Table 4, as described before (24).

Statistical analysis: Genotype and allelic frequencies of polymorphisms at each site were determined. To assess differences in the distribution of polymorphisms at each site between patients and controls, the Chi square test was used. The Mann-Whitney U test was used to evaluate the relationship between disease severity and genotypes, and between blood TP concentrations and genotypes for each polymorphism. All statistical analyses were done using SPSS, version 11.01.

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