

Xenobiotic/drug metabolizing enzyme and TP53 polymorphisms and clinical outcome in advanced nonsmall cell lung cancer patients

Volkan KARACAOĞLAN^{1,2}, Ahmet Oğuz ADA¹, Serdar BİLGİN¹, Guzide Tuğba ÇETİNKAYA¹, Emre SOYDAŞ¹, Celalettin Semih KUNAK³, Sibel Meryem ALPAR^{4,5}, Meral GÜLHAN^{4,6}, Mümtaz İŞCAN^{1,*}

¹Department of Toxicology, Faculty of Pharmacy, Ankara University, Ankara, Turkey

²Department of Toxicology, Faculty of Pharmacy, Bülent Ecevit University, Zonguldak, Turkey

³Department of Pharmacology, Faculty of Medicine, Ordu University, Ordu, Turkey

⁴Atatürk Pulmonary Diseases and Thoracic Surgery Hospital, Ankara, Turkey

⁵Lokman Hekim Hospital, Sincan, Ankara, Turkey

⁶Department of Chest Diseases, Ridvan Ege Hospital, Ufuk University, Ankara, Turkey

Received: 13.02.2016 • Accepted/Published Online: 11.09.2016 • Final Version: 18.04.2017

Background/aim: The association between polymorphisms of xenobiotic/drug metabolizing enzymes and TP53 and response to chemotherapy and survival of patients with nonsmall cell lung cancer (NSCLC) are limited and inconclusive. In this study, *CYP2E1*5B*, *CYP2E1*6*, *CYP2E1*7B*, *GSTO1* (A140D), and *TP53* (Arg72Pro) polymorphisms and response to platinum-based chemotherapy and survival in 137 advanced stage NSCLC patients were investigated.

Materials and methods: Genetic polymorphism analyses were determined by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP).

Results: The patients with *TP53* Pro/Pro variant were more likely to be resistant to chemotherapy than those with Arg/Arg variants with marginal significance ($P = 0.066$). We also analyzed these gene variants in combination with *CYP1A1* (Ile462Val), *CYP1B1* (Asn453Ser), *GSTM1*, *GSTP1* exon 5 (Ile105Val), and *GSTP1* exon 6 (Ala114Val) and *GSTT1* polymorphic genes that we have previously genotyped in the same patients (Ada et al., *Neoplasma*, 57, 512-527, 2010). The multivariate analysis revealed that adjusted hazard ratio (HR) of death of the combined variant genotypes of *TP53* (Arg72Pro, Pro72Pro) and *CYP1A1* (Ile462Val, Val462Val) increased significantly as compared to wild-type genotypes (HR, 6.03; 95% CI, 1.39–26.04, $P = 0.016$).

Conclusion: These results show that combined variant genotypes of *TP53* (Arg72Pro, Pro72Pro) and *CYP1A1* (Ile/Val, Val/Val) are associated with worsening of survival in NSCLC patients.

Key words: Xenobiotic/drug metabolizing enzymes, TP53, polymorphisms, response to chemotherapy, survival, nonsmall cell lung cancer

1. Introduction

Lung cancer is the worldwide leading cause of cancer mortality (1). Nonsmall cell lung cancer (NSCLC) patients represent the majority of lung cancer cases and they are mainly treated with standard platinum-based chemotherapy (2). However, the poor response and a great interindividual variety in response to this chemotherapy treatment occur among these patients (3). Thus, the reasons behind the failure and interindividual variety of response to chemotherapy and thus possibly poorer survival in these patients are very important.

The majority of lung cancer patients are smokers (4). Cigarette smoke is known to increase the carcinogen DNA-adduct levels, which in turn form aggressive tumors

by mutating and thus inactivating tumor suppressor genes, such as *TP53*, and thereby decrease the survival rates of patients with NSCLC (5,6).

Metabolic activation of N-nitrosamines such as nicotine-derived nitrosamine ketone (NNK), benzene, and vinyl chloride in cigarette smoke to mutagenic and carcinogenic metabolites are mediated by *CYP2E1* (7). In addition, *CYP2E1* also plays a role in the metabolism of a number of chemotherapeutic agents and thus is involved in drug resistance (8). The expression of *CYP2E1* has also been found to be increased in lung cancer (9,10). The most common alleles and polymorphisms of *CYP2E1* are *CYP2E1*5B* (RsaI/PstI C1053T/C1293C) (11,12) and *CYP2E1*6* (DraI T7632A) (13). The variant alleles

* Correspondence: iscan@pharmacy.ankara.edu.tr

have been shown to lower activities of the corresponding enzymes (11,12). In addition, the *CYP2E1* gene has also the *CYP2E1*7B* allele (14) but no information is available on its possible activity alteration. Several studies have also shown the existence of an association between lung cancer and *CYP2E1*5B* (15,16) and *CYP2E1*6* (13,15) polymorphisms in various populations.

On the other hand, one of the members of glutathione S-transferase (GST) family, GST Omega 1 (*GSTO1*), plays a role in apoptosis (17) and is a potential reservoir of intracellular glutathione (GSH), which protects against cellular oxidative stress (18). The protective role against cell toxicity can be weakened if the enzyme activity is reduced, but the findings related to the *GSTO1* gene polymorphism Ala140Asp (A140D) are still inconclusive (19,20). Recent studies have established an association between the *GSTO1* (A140D) gene polymorphism and increased risk of several carcinomas such as breast and hepatocellular carcinoma (21) but not with lung or colorectal cancers (21,22).

The *TP53* gene is a well-known tumor suppressor gene that regulates cell-cycle arrest, DNA repair, and apoptosis in response to cellular stress including chemotherapy (23). Thus, normal activity of TP53 is necessary for the sensitivity of the cancer cells to chemotherapeutics and thus the inhibition of TP53 can lead to chemoresistance (24). Tobacco-specific carcinogenic compounds have also been shown to cause mutations in the *TP53* gene (25). Several functional SNPs occur in the *TP53* gene and the most frequently studied is the polymorphism *TP53* (Arg72Pro), the variant allele being altered, decreasing the TP53 activity in apoptosis (26,27). Emerging evidence, although inconclusive, has shown that *TP53* (Arg72Pro) polymorphism is not only associated with lung cancer risk but also influences patient response to platinum-based chemotherapy and survival (28–33). Furthermore, associations have also been shown between polymorphisms of some CYP genes such as the *CYP2E1* or *CYP1A1* and *TP53* gene in NSCLC (5,6,9,34,35).

All this information is necessary and important in terms of determining the predictive and prognostic significances of these genotypes of NSCLC patients, leading to the availability of the tool needed by clinicians to individualize therapies and accurately predict survival. However, a limited number of molecular epidemiological studies, with controversial results, to date have considered determining the role of *CYP2E1*5B* (34–36), *CYP2E1*6* (37), and *TP53* (Arg72Pro) (28–31,38,39) polymorphisms in this regard. In addition, to the best of our knowledge, no information is available with respect to *CYP2E1*7B* and *GSTO1* (A140D) polymorphisms and their overall combined impact on clinical outcome in NSCLC.

In the present study, we aimed to determine the association either alone or in combination between the

*CYP2E1*5B*, *CYP2E1*6*, *CYP2E1*7B*, *GSTO1* (A140D), and *TP53* (Arg72Pro) polymorphisms and response to platinum-based chemotherapy and survival in advanced stage NSCLC patients. Given the complexity of the pathways of drugs/pro-carcinogens and the possible interactions between encoding activation/inactivation enzymes and TP53 protein that might have cooperative impact on outcome of NSCLC patients treated with platinum-based chemotherapy, we further analyzed the possible interactions combining these gene polymorphisms with *CYP1A1* (Ile462Val), *CYP1B1* (Asn453Ser), *GSTM1*, *GSTP1* (Ile105Val), *GSTP1* (Ala114Val), and *GSTT1* gene polymorphisms that we previously genotyped in the same patients (40).

2. Materials and methods

2.1. Patients

In total, 137 patients of mean age 56 ± 9 (mean \pm SD; range: 34–75) who had a histological diagnosis of primary NSCLC with stages III or IV and who were treated with platinum-based chemotherapy were enrolled in this study; 125 of these patients were male, with a mean age of 56 ± 9 (mean \pm SD; range: 34–75), and 12 were female, with a mean age of 58 ± 8 (mean \pm SD; range: 44–69). All patients were recruited from Atatürk Pulmonary Diseases and Thoracic Surgery Hospital from February 2002 to November 2005. All patients provided written informed consent and the study was approved by the Medical Ethics Board of Atatürk Pulmonary Diseases and Thoracic Surgery Hospital. Clinical information and the chemotherapy regimen of patients and the evaluation of the effect of chemotherapy have been described in detail elsewhere (40,41). The responder group consisted of patients with complete response (CR) and partial response (PR) and the nonresponsive group consisted of patients with stable disease (SD) and progressive disease (PD).

2.2. Genotyping procedure

Lymphocyte DNA was isolated from the patients using a Promega genomic DNA purification kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Genetic polymorphism analyses were conducted by PCR-RFLP method. PCR master mixes were obtained from Qiagen (Hilden, Germany). Restriction enzymes were purchased from NEB (Ipswich, MA, USA). *CYP2E1*5B* polymorphism was determined by the method of Hayashi et al. (11). *CYP2E1*6* polymorphism was determined by the method of Kato et al. (42). *CYP2E1*7B* polymorphism was determined using the method of Yang et al. (43). Genetic polymorphism analysis for the *GSTO1* (A140D) was determined by the method described by Marahatta et al. (21). The *TP53* (Arg72 Pro) gene polymorphism was determined by the method of Hu et al. (30). For quality control, the laboratory personnel

were blinded to the source of each DNA specimen and a random 10% of the samples were repeated with 100% concordance. Two authors reviewed independently 100% of the agarose gels and genotype data entry.

2.3. Statistical analysis

Chi-square analysis and Fisher's exact tests were used to compare the distribution of genotypes between subgroups and response to chemotherapy. We calculated survival as the period from diagnosis to the date of death or the date of last follow-up for each patient. Overall survival in relation to *CYP*, *GST*, and *TP53* genotypes was evaluated by the Kaplan–Meier survival function and log-rank tests. Hazard ratios (HRs) were estimated from a multivariate Cox proportional hazards model with adjustment for age, sex, smoking status, chemotherapy regimen, tumor stage, and tumor histology. Only P values < 0.05 were considered significant. SPSS (SPSS, Inc., Chicago, IL, USA) was used for the statistical analysis.

3. Results

Characteristics of the 137 patients at diagnosis are provided in Table 1. Among the 137 patients, 42 (31%) of them responded to the platinum-based first-line chemotherapy, whereas 95 (69%) of them did not. When the distributions of response to chemotherapy according to patient characteristics were evaluated they were not found to be related to age, sex, tumor histology, stage at diagnosis, chemotherapy regimen, or smoking status ($P > 0.05$, data not shown).

The distributions of the genotypes (either alone or in combination) according to patient characteristics were also evaluated and were not observed to be related to age, sex, tumor histology, stage at diagnosis, or smoking status ($P > 0.05$, data not shown).

Although no significant associations were noted between the gene polymorphisms alone or in combination and response to chemotherapy, patients with the *TP53*

Table 1. Characteristics of 137 NSCLC patients.

Characteristics		Number of patients
Total		137
Age		
	≤50	40
	51–60	47
	≥61	50
Sex		
	Male	125
	Female	12
Histology		
	Squamous cell carcinoma	49
	Adenocarcinoma	48
	Unspecified nonsmall cell lung cancer	40
Stage at diagnosis		
	Stage III	60
	Stage IV	77
Chemotherapy ^a		
	Platinum + Etoposide ^b	86
	Platinum + others ^c	51
Smoking status		
	Never	13
	Current	85
	Former	39

^aThe chemotherapy regimens are detailed previously (40)

^bCisplatin + Etoposide

^cCisplatin + Gemcitabine, Cisplatin + Docetaxel, Cisplatin + Vinorelbine, Cisplatin + Paclitaxel, Carboplatin + Paclitaxel

Pro/Pro variant were more likely to be resistant to chemotherapy than those with Arg/Arg variants (100% vs. 66%) or with Arg/Arg and Arg/Pro variants (100% vs. 68%), with marginal significance ($P = 0.066$ and $P = 0.071$, respectively) (Table 2). No significant associations were noted between the responses of the genotypes (either alone or in combination) and age, sex, smoking status, chemotherapy regimen, tumor stage, or histology ($P > 0.05$, data not shown).

The Kaplan–Meier survival functions for overall survival according to the genotypes (either alone or in combination) were analyzed. In total, 58 (42%) deaths were observed during follow-up. Among the genotypes either alone or in combination, there was no significant association between *CYP2E1*, *GSTO1* (A140D), and *TP53* genotypes and Kaplan–Meier function survival rates ($P > 0.05$, data not shown). We also investigated the possible interactions for combining these genes with polymorphic genes of CYPs (*CYP1A1* and *CYP1B1*) and GSTs (*GSTM1*, *GSTP1*, and *GSTT1*) that we previously genotyped in these patients (40). However, while in the previous study (40) the number of the patients was 138, in the present study the number of patients enrolled was 137. We enrolled 137 patients because one patient's DNA had run out. Therefore, we statistically recalculated the parameters of 137 patients of the previous study, excluding the patient's data whose DNA had finished. No significant associations were noted between the combined genotypes and responses to chemotherapy. However, only two of them revealed a remarkably altered survival period. The patients who had both variant genotypes of *TP53* (Arg/Pro, Pro/Pro) and *CYP1A1* (Ile/Val, Val/Val) had shorter survival (median, 15.6 months) compared to those with wild-type genotypes (median, 19.4 months) ($P = 0.480$) (data not shown). Likewise, the patients who had variant genotypes of both *TP53* (Arg/Pro, Pro/Pro) and *GSTO1* (A/D, D/D) had shorter survival (median, 18.4 months) compared to those with wild-type genotypes (median, 22.7 months) ($P = 0.560$) (data not shown).

The distributions of *CYP2E1*, *GSTO1*, and *TP53* genotypes (either alone or in combinations) and survival of the NSCLC patients are shown in Tables 3 and 4. However, due to the very limited number of patients with null and/or variant genotypes, only the genotype combinations that were available for statistical analysis are given in Table 4. Overall multivariate analysis revealed no significant HR of death associated with the genotype combinations. When we analyzed the possible interactions combining these gene polymorphisms with *CYP* and *GST* gene polymorphisms that we previously genotyped in the same patients (40), one of the genotype combinations showed a remarkably significant association with HR of death. The death risk of combined variant genotypes of *TP53* (Arg/Pro, Pro/Pro) and *CYP1A1* (Ile/Val, Val/Val) increased significantly as compared to wild-type genotypes (HR, 6.03; 95% CI, 1.39–26.04, $P = 0.016$) (Table 4). The other genotype combinations that showed remarkable but not significant increases in HR of death were *CYP2E1**7B (*1A/*7B) and *TP53* (Arg/Pro, Pro/Pro) (HR, 2.70; 95% CI, 0.80–9.08, $P = 0.108$) and *GSTO1* (A/D, D/D) and *TP53* (Arg/Pro, Pro/Pro) (HR, 2.52; 95% CI, 0.75–8.49, $P = 0.137$).

4. Discussion

To the best of our knowledge this is the first study investigating the joint effect of *TP53* (Arg72Pro) and the aforementioned *CYP* and *GST* polymorphisms on the clinical outcome of NSCLC patients with platinum-based chemotherapy. In the current study, we found that the *TP53* Pro/Pro genotype was likely to be resistant to platinum-based chemotherapy, with marginal significance ($P = 0.066$), but unlikely to predict the survival. Our data also indicated that the combined polymorphisms of *TP53* (Arg72Pro) and *CYP1A1* (Ile 462Val) were likely to play a role in the prognosis of NSCLC patients treated with platinum-based chemotherapy.

In regard to *CYP2E1* polymorphisms and survival in lung cancer, only a few studies exist and their results are rather contradictory. For example, the studies on *CYP2E1**5B

Table 2. The distributions of *TP53* genotypes according to response to chemotherapy.

Genotype	Response to chemotherapy		
	Responder	Nonresponder	P-value
<i>TP53</i> (Arg72Arg)	14	27	0.066
<i>TP53</i> (Pro72Pro)	0	7	
<i>TP53</i> (Arg72Arg + Arg72Pro)	42	88	0.071
<i>TP53</i> (Pro72Pro)	0	7	
<i>TP53</i> (Arg72Arg)	14	27	0.563
<i>TP53</i> (Arg72Pro + Pro72Pro)	28	68	

Table 3. CYP, GST, and TP53 genotypes (alone) and survival of NSCLC patients.

Genotype	n	Overall survival	
		HR (95% CI) ^a	P-value
<i>CYP2E1*5B</i> (*1A/*1A)	132	1	
<i>CYP2E1*5B</i> (*1A/*5B)	5	1.23 (0.25–6.06)	0.801
<i>CYP2E1*6</i> (*1A/*1A)	121	1	
<i>CYP2E1*6</i> (*1A/*6)	16	1.36 (0.63–2.92)	0.432
<i>CYP2E1*7B</i> (*1A/*1A)	124	1	
<i>CYP2E1*7B</i> (*1A/*7B)	13	1.02 (0.41–2.53)	0.958
<i>GSTO1</i> (A/A)	70	1	
<i>GSTO1</i> (A/D+D/D)	67	1.04 (0.65–1.66)	0.875
<i>TP53</i> (Arg/Arg)	41	1	
<i>TP53</i> (Arg/Pro)	89	1.27 (0.02–2.57)	0.226
<i>TP53</i> (Arg/Arg)	41	1	
<i>TP53</i> (Pro/Pro)	7	0.22 (0.69–3.07)	0.318
<i>TP53</i> (Arg/Arg)	41	1	
<i>TP53</i> (Arg/Pro+Pro/Pro)	96	1.14 (0.62–2.11)	0.667

^aHR: hazard ratio, 95% CI: 95% confidence interval.

Variant genotype compared to wild-type genotype. HR and 95% CI values were determined by using Cox proportional hazards model that was adjusted for age, sex, tumor histology, tumor stage, smoking status, chemotherapy regimen, and response to chemotherapy.

are rather conflicting. While Oyama et al. (35) found an increase in survival in mutant allele carriers, Haque et al. (34) observed a shorter survival in mutant carriers and Li et al. (36) did not find any association between this *CYP* gene polymorphism and survival in NSCLC. Przygodzki et al. (37) could not find any significant association between *CYP2E1*6* polymorphisms and survival in NSCLC patients. Moreover, almost no information is available with respect to the relationship between these polymorphisms and response to chemotherapy in NSCLC patients. The only data in this regard were recently provided by Li et al. (36), who did not observe any significant association between *CYP2E1*5B* polymorphism and response to chemotherapy in NSCLC. Thus, based on the previously reported results on *CYP2E1*5B*, our results are in line with the findings given by Li et al. (36) in regard to both response to chemotherapy and survival but in contrast to those of Oyama et al. (35) and Haque et al. (34) in respect to survival. Our findings in regard to *CYP2E1*6* polymorphisms on survival also coincided with the results of Przygodzki et al. (37). The *CYP2E1*7B* polymorphism appeared to have no effect on the prognosis of NSCLC. The studied *CYP2E1* polymorphisms either alone or in

combination are unlikely to play a role in the prognosis of NSCLC. The reasons for the inconsistent results of the *CYP2E1*5B* polymorphism on survival among these studies remain to be explored in further studies.

Previous reports suggested that *GSTO1* (A140D) polymorphisms might be associated with lung cancer in smokers (44,45). However, we could not find any association in our Turkish population (22). In the current study, *GSTO1* (A140D) polymorphism alone has been shown to have no effect on NSCLC prognosis.

The 72 Pro variant was shown to have less apoptotic potential than the 72Arg, rendering this polymorphism one of the most frequently studied variations in the P53 pathway (26,27). Although *TP53* (Arg72Pro) polymorphism has been shown to affect the prognosis of various cancers (46,47), findings for NSCLC is still inconclusive and controversial (28–31,38,39). Our findings with respect to resistance to chemotherapy are similar to the results of the study by Han et al. (29), who observed the variant allele was resistant to first-line chemotherapy in NSCLC. Among our nonresponsive patients carrying the 72 Pro variant allele, 3 of them were treated with platinum and etoposide and 4 of them were treated with

Table 4. CYP, GST, and TP53 genotypes (in combination) and survival of NSCLC patients.

Genotype	n	Overall survival	
		HR (95% CI) ^a	P-value
<i>CYP2E1</i> *5B (*1A/*1A)+ <i>TP53</i> (Arg/Arg)	39	1	
<i>CYP2E1</i> *5B (*1A/*5B)+ <i>TP53</i> (Arg/Pro+Pro/Pro)	3	1.73 (0.16–18.53)	0.649
<i>CYP2E1</i> *6 (*1A/*1A)+ <i>TP53</i> (Arg/Arg)	37	1	
<i>CYP2E1</i> *6 (*1A/*6) + <i>TP53</i> (Arg/Pro+Pro/Pro)	12	1.81 (0.54–6.06)	0.336
<i>CYP2E1</i> *7B (*1A/*1A)+ <i>TP53</i> (Arg/Arg)	40	1	
<i>CYP2E1</i> *7B(*1A/*7B) + <i>TP53</i> (Arg/Pro+Pro/Pro)	12	2.70 (0.80–9.08)	0.108
<i>GSTO1</i> (A/A) + <i>TP53</i> (Arg/Arg)	17	1	
<i>GSTO1</i> (A/D+D/D) + <i>TP53</i> (Arg/Pro+Pro/Pro)	43	2.52 (0.75–8.49)	0.137
<i>TP53</i> (Arg/Arg) + <i>CYP1A1</i> (Ile/Ile)	31	1	
<i>TP53</i> (Arg/Pro+Pro/Pro) + <i>CYP1A1</i> (Ile/Val+Val/Val)	14	6.03 (1.39–26.04)	0.016
<i>TP53</i> (Arg/Arg) + <i>CYP1B1</i> (Asn/Asn)	26	1	
<i>TP53</i> (Arg/Pro+Pro/Pro) + <i>CYP1B1</i> (Asn/Ser+Ser/Ser)	27	1.22 (0.45–3.28)	0.695
<i>TP53</i> (Arg/Arg) + <i>GSTM1</i> positive	17	1	
<i>TP53</i> (Arg/Pro+Pro/Pro) + <i>GSTM1</i> null	56	0.90 (0.34–2.37)	0.834
<i>TP53</i> (Arg/Arg) + <i>GSTT1</i> positive	29	1	
<i>TP53</i> (Arg/Pro+Pro/Pro) + <i>GSTT1</i> null	25	1.72 (0.52–5.62)	0.371
<i>TP53</i> (Arg/Arg) + <i>GSTP1</i> exon 5 (Ile/Ile)	24	1	
<i>TP53</i> (Arg/Pro+Pro/Pro) + <i>GSTP1</i> exon 5 (Ile/Val+Val/Val)	34	1.40 (0.51–3.83)	0.513
<i>TP53</i> (Arg/Arg) + <i>GSTP1</i> exon 6 (Ala/Ala)	30	1	
<i>TP53</i> (Arg/Pro+Pro/Pro) + <i>GSTP1</i> exon 6 (Ala/Val+Val/Val)	19	0.65 (0.16–2.73)	0.562

^aHR: hazard ratio, 95% CI: 95% confidence interval.

Null or variant genotype compared to present or wild-type genotype. HR and 95% CI values were determined by using Cox proportional hazards model that was adjusted for age, sex, tumor histology, tumor stage, smoking status, chemotherapy regimen, and response to chemotherapy.

platinum and other chemotherapeutics. In the study by Han et al. (29), the nonresponsive patients carrying the 72 Pro variant allele were resistant to an irinotecan plus cisplatin regimen. These findings seem to reveal that this polymorphism is predictive for primary resistance especially to these chemotherapeutic drugs.

With respect to overall survival our results are in line with the findings of several investigators (28–30,38) while in contrast to those of others (31,39). At this stage, the reasons for the inconsistent results among all these studies, including ours, are not clear. Nevertheless, methodological and statistical discrepancies may, in part, account for the lack of consistent findings.

On the other hand, the lack of association between *CYP2E1* or *GSTO1* genotypes and response to chemotherapy observed in the current study is likely to show that these polymorphisms are not functioning as a predictor of response to these two distinct platinum-

based chemotherapy regimens (platinum and etoposide or platinum and other chemotherapeutics).

Recent studies have demonstrated that the simultaneous analysis of such gene polymorphisms may correlate well with the clinical outcome better than the single polymorphism studies. For example, the combined variant *CYP1A1* (Ile462Val) and *GSTM1* null genotype was associated with better response to chemotherapy but not with survival in lung cancer (36). Our previous study in NSCLC patients also revealed *CYP1A1* and *GSTP1* exon 5 variant alleles or *CYP1B1* and *GSTP1* exon 5 variant alleles had notable trends toward worsening of survival, whereas better survival was noted with combined *GSTP1* exon 5 and *GSTP1* exon 6 variant alleles (40).

In the present study, the combined variant genotypes of *TP53* (Arg/Pro, Pro/Pro) and *CYP1A1* (Ile/Val, Val/Val) were determined to play a role in the prognosis, a prognostic of worse survival, in patients with advanced

NSCLC. Likewise, previous studies demonstrated that *CYP1A1* gene Msp1 mutation carrier NSCLC patients had higher rates of *TP53* mutations and variant allele carriers of the *CYP1A1* (Msp1) gene had shorter survival compared to those of wild-type genotypes in advanced NSCLC (5,6,10,35). The variant alleles of *CYP1A1* such as *CYP1A1*2A* (Msp1) and *CYP1A1*2C* (Ile462Val) have higher enzyme activities (48,49). Positive associations have also been observed between these polymorphisms and benzo(a)pyrene 7,8-9,10 diol epoxide (BaPDE)-DNA adduct levels in the lungs of smokers or increase in cancer risk in various populations (15,50). Thus, the observed finding seems to be conceivable as the *CYP1A1*2C* gene variant elevates enzyme activity, which leads to more tobacco-specific PAH-activated carcinogenic/mutagenic e.g. BaPDE-DNA adducts, which in turn cause the formation of aggressive tumors by mutating and thus

inactivating tumor suppressor gene *TP53*, and thereby decreasing the survival rates of patients with NSCLC. In addition, in the current study, the combined *CYP2E1*7B* and *TP53* variant alleles and *GSTO1* and *TP53* variant alleles demonstrated notable trends toward worsening survival.

In summary we have demonstrated that the combined variant genotypes of *TP53* (Arg/Pro, Pro/Pro) and *CYP1A1* (Ile/Val, Val/Val) are associated with worsening survival in advanced NSCLC patients treated with platinum-based chemotherapy. However, additional studies are required to confirm our finding.

Acknowledgment

This research was supported by the grants from Research Funds of Ankara University (nos: 2008-08-03-006HPD and 10A3336002).

References

1. Howe HL, Wingo PA, Thun MJ, Ries LA, Rosenberg HM, Feigal EG, Edwards BK. Annual report to the nation on the status of cancer (1973 through 1998), featuring cancers with recent increasing trends. *J Natl Cancer Inst* 2001; 93: 824-842.
2. Bunn PA. Chemotherapy for advanced non-small-cell lung cancer: who, what, when, why? *J Clin Oncol* 2002; 20: 23S-33S.
3. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. *Mayo Clin Proc* 2008; 83: 584-594.
4. Siemiatycki J, Krewski D, Franco E, Kaiserman M. Associations between cigarette smoking and each of 21 types of cancer: a multi-site case-control study. *Int J Epidemiol* 1995; 24: 504-514.
5. Goto I, Yoneda S, Yamamoto M, Kawajiri K. Prognostic significance of germ line polymorphisms of the *CYP1A1* and glutathione S-transferase genes in patients with non-small cell lung cancer. *Cancer Res* 1996; 56: 3725-3730.
6. Kawajiri K, Eguchi H, Nakachi K, Sekiya T, Yamamoto M. Association of *CYP1A1* germ line polymorphisms with mutations of the *p53* gene in lung cancer. *Cancer Res* 1996; 56: 72-76.
7. Guengerich FP, Kim DH, Iwasaki M. Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem Res Toxicol* 1991; 4: 168-179.
8. Michael M, Doherty MM. Drug metabolism by tumours: its nature, relevance and therapeutic implications. *Expert Opin Drug Metab Toxicol* 2007; 3: 783-803.
9. Oyama T, Kawamoto T, Mizoue T, Sugio K, Kodama Y, Mitsudomi T, Yasumoto K. Cytochrome P450 2E1 polymorphism as a risk factor for lung cancer: in relation to *p53* gene mutation. *Anticancer Res* 1997; 17: 583-587.
10. Oyama T, Sugio K, Uramoto H, Iwata T, Onitsuka T, Isse T, Nozoe T, Kagawa N, Yasumoto K, Kawamoto T. Increased cytochrome P450 and aryl hydrocarbon receptor in bronchial epithelium of heavy smokers with non-small cell lung carcinoma carries a poor prognosis. *Front Biosci* 2007; 12: 4497-4503.
11. Hayashi S, Watanabe J, Kawajiri K. Genetic polymorphisms in the 5'-flanking region change transcriptional regulation of the human cytochrome P450IIE1 gene. *J Biochem* 1991; 110: 559-565.
12. Lucas D, Ménez C, Girre C, Berthou F, Bodénez P, Joannet I, Hispard E, Bardou LG, Ménez JF. Cytochrome P450 2E1 genotype and chlorzoxazone metabolism in healthy and alcoholic Caucasian subjects. *Pharmacogenet Genomics* 1995; 5: 298-304.
13. Uematsu F, Kikuchi H, Motomiya M, Abe T, Sagami I, Ohmachi T, Wakui A, Kanamaru R, Watanabe M. Association between restriction fragment length polymorphism of the human cytochrome P450IIE1 gene and susceptibility to lung cancer. *Cancer Sci* 1991; 82: 254-256.
14. Fairbrother KS, Grove J, de Waziers I, Steimel DT, Day CP, Crespi CL, Daly AK. Detection and characterization of novel polymorphisms in the *CYP2E1* gene. *Pharmacogenet Genomics* 1998; 8: 543-552.
15. Le Marchand L, Sivaraman L, Pierce L, Seifried A, Lum A, Wilkens LR, Lau AF. Associations of *CYP1A1*, *GSTM1*, and *CYP2E1* polymorphisms with lung cancer suggest cell type specificities to tobacco carcinogens. *Cancer Res* 1998; 58: 4858-4863.
16. Wang SL, Lee H, Chen KW, Tsai KJ, Chen CY, Lin P. Cytochrome P4502E1 genetic polymorphisms and lung cancer in a Taiwanese population. *Lung Cancer* 1999; 26: 27-34.

17. Wang L, Xu J, Ji C, Gu S, Lv Y, Li S, Xu Y, Xie Y, Mao Y. Cloning, expression and characterization of human glutathione S-transferase Omega 2. *Int J Mol Med* 2005; 16: 19-27.
18. Board PG. The omega-class glutathione transferases: structure, function, and genetics. *Drug Metab Rev* 2011; 43: 226-235.
19. Tanaka-Kagawa T, Jinno H, Hasegawa T, Makino Y, Seko Y, Hanioka N, Ando M. Functional characterization of two variant human GSTO 1-1s (Ala140Asp and Thr217Asn). *Biochem Biophys Res Commun* 2003; 301: 516-520.
20. Whitbread AK, Tetlow N, Eyre HJ, Sutherland GR, Board PG. Characterization of the human Omega class glutathione transferase genes and associated polymorphisms. *Pharmacogenetics* 2003; 13: 131-144.
21. Marahatta SB, Punyarit P, Bhudisawasdi V, Paupairoj A, Wongkham S, Petmitr S. Polymorphism of glutathione S-transferase omega gene and risk of cancer. *Cancer Lett* 2006; 236: 276-281.
22. Ada TG, Ada AO, Kunak SC, Alpar S, Gulhan M, Iscan M. Association between glutathione S-transferase omega 1 A140D polymorphism in the Turkish population and susceptibility to non-small cell lung cancer. *Arh Hig Rada Toksikol* 2013; 64: 61-67.
23. Hrstka R, Coates PJ, Vojtesek B. Polymorphisms in p53 and the p53 pathway: roles in cancer susceptibility and response to treatment. *J Cell Mol Med* 2009; 13: 440-453.
24. Malkin D, Jolly KW, Barbier N, Look AT, Friend SH, Gebhardt MC, Andersen TI, Borresen AL, Li FP, Garber J, Strong LC. Germline mutations of the p53 tumor-suppressor gene in children and young adults with second malignant neoplasms. *N Engl J Med* 1992; 326: 1309-1315.
25. Denissenko MF, Pao A, Tang M, Pfeifer GP. Preferential formation of benzo [a] pyrene adducts at lung cancer mutational hotspots in P53. *Science* 1996; 274: 430-432.
26. Dumont P, Leu JI, Della Pietra AC, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. *Nat Genet* 2003; 33: 357-365.
27. Thomas M, Kalita A, Labrecque S, Pim D, Banks L, Matlashewski G. Two polymorphic variants of wild-type p53 differ biochemically and biologically. *Mol Cell Biol* 1999; 19: 1092-1100.
28. Chua HW, Ng D, Choo S, Lum SS, Li H, Soh LY, Sabapathy K, Seow A. Effect of MDM2 SNP309 and p53 codon 72 polymorphisms on lung cancer risk and survival among non-smoking Chinese women in Singapore. *BMC Cancer* 2010; 10: 88.
29. Han JY, Lee GK, Jang DH, Lee SY, Lee JS. Association of p53 codon 72 polymorphism and MDM2 SNP309 with clinical outcome of advanced nonsmall cell lung cancer. *Cancer*. 2008; 113: 799-807.
30. Hu Y, McDermott MP, Ahrendt SA. The p53 codon 72 proline allele is associated with p53 gene mutations in non-small cell lung cancer. *Clin Cancer Res* 2005; 11: 2502-2509.
31. Liu L, Wu C, Wang Y, Zhong R, Duan S, Wei S, Lin S, Zhang X, Tan W, Yu D. Combined effect of genetic polymorphisms in P53, P73, and MDM2 on non-small cell lung cancer survival. *J Thorac Oncol* 2011; 6: 1793-1800.
32. Muller M, Schleithoff ES, Stremmel W, Melino G, Krammer PH, Schilling T. One, two, three – p53, p63, p73 and chemosensitivity. *Drug Resist Updat* 2006; 9: 288-306.
33. Wu X, Zhao H, Amos CI, Shete S, Makan N, Hong WK, Kadlubar FF, Spitz MR. p53 genotypes and haplotypes associated with lung cancer susceptibility and ethnicity. *J Natl Cancer Inst* 2002; 94: 681-690.
34. Haque AK, Au W, Cajas-Salazar N, Khan S, Ginzel AW, Jones DV, Zwischenberger JB, Xie J. CYP2E1 polymorphism, cigarette smoking, p53 expression, and survival in non-small cell lung cancer: a long term follow-up study. *Appl Immunohistochem Mol Morphol* 2004; 12: 315-322.
35. Oyama T, Matsumoto A, Isse T, Kim YD, Ozaki S, Osaki T, Sugio K, Yasumoto K, Kawamoto T. Evidence-based prevention (EBP): approach to lung cancer prevention based on cytochrome 1A1 and cytochrome 2E1 polymorphism. *Anticancer Res* 2003; 23: 1731-1737.
36. Li W, Yue W, Zhang L, Zhao X, Ma L, Yang X, Zhang C, Wang Y, Gu M. Polymorphisms in GSTM1, CYP1A1, CYP2E1, and CYP2D6 are associated with susceptibility and chemotherapy response in non-small-cell lung cancer patients. *Lung* 2012; 190: 91-98.
37. Przygodzki RM, Bennett WP, Guinee Jr DG, Khan MA, Freedman A, Shields PG, Travis WD, Jett JR, Tazelaar H, Pairolero P. p53 mutation spectrum in relation to GSTM1, CYP1A1 and CYP2E1 in surgically treated patients with non-small cell lung cancer. *Pharmacogenet Genomics* 1998; 8: 503-512.
38. Matakidou A, El Galta R, Webb EL, Rudd MF, Bridle H, Eisen T, Houlston RS. Lack of evidence that p53 Arg72Pro influences lung cancer prognosis: an analysis of survival in 619 female patients. *Lung Cancer* 2007; 57: 207-212.
39. Sreeja L, Syamala V, Hariharan S, Syamala VS, Raveendran PB, Sivanandan C, Madhavan J, Ankathil R. Glutathione S-transferase M1, T1 and P1 polymorphisms: susceptibility and outcome in lung cancer patients. *J Exp Ther Oncol* 2007; 7: 73-85.
40. Ada A, Kunak S, Hancer F, Bilgen S, Suzen S, Alpar S, Gulhan M, Kurt B, Iscan M. CYP and GST polymorphisms and survival in advanced non-small cell lung cancer patients. *Neoplasma* 2010; 57: 512-521.
41. Ada AO, Kunak SC, Hancer F, Soydas E, Alpar S, Gulhan M, Iscan M. Association between GSTM1, GSTT1, and GSTP1 polymorphisms and lung cancer risk in a Turkish population. *Mol Biol Rep* 2012; 39: 5985-5993.
42. Kato S, Shields PG, Caporaso NE, Sugimura H, Trivers GE, Tucker MA, Trump BF, Weston A, Harris CC. Analysis of cytochrome P450 2E1 genetic polymorphisms in relation to human lung cancer. *Cancer Epidemiol Biomarkers Prev* 1994; 3: 515-518.

43. Yang B, O'Reilly DA, Demaine AG, Kingsnorth AN. Study of polymorphisms in the CYP2E1 gene in patients with alcoholic pancreatitis. *Alcohol* 2001; 23: 91-97.
44. Hu YC, Sidransky D, Ahrendt SA. Molecular detection approaches for smoking associated tumors. *Oncogene* 2002; 21: 7289-7297.
45. Yanbaeva DG, Wouters EF, Dentener MA, Spruit MA, Reynaert NL. Association of glutathione-S-transferase omega haplotypes with susceptibility to chronic obstructive pulmonary disease. *Free Radic Res* 2009; 43: 738-743.
46. Toyama T, Zhang Z, Nishio M, Hamaguchi M, Kondo N, Iwase H, Iwata H, Takahashi S, Yamashita H, Fujii Y. Association of TP53 codon 72 polymorphism and the outcome of adjuvant therapy in breast cancer patients. *Breast Cancer Res* 2007; 9: R34.
47. Yamasaki M, Miyata H, Fujiwara Y, Takiguchi S, Nakajima K, Nishida T, Yasuda T, Matsuyama J, Mori M, Doki Y. p53 genotype predicts response to chemotherapy in patients with squamous cell carcinoma of the esophagus. *Ann Surg Oncol* 2010; 17: 634-642.
48. Cascorbi I, Brockmoller J, Roots I. A C4887A polymorphism in exon 7 of human CYP1A1: population frequency, mutation linkages, and impact on lung cancer susceptibility. *Cancer Res* 1996; 56: 4965-4969.
49. Hayashi S, Watanabe J, Nakachi K, Kawajiri K. Genetic linkage of lung cancer-associated MspI polymorphisms with amino acid replacement in the heme binding region of the human cytochrome P450IA1 gene. *J Biochem* 1991; 110: 407-411.
50. Bartsch H, Castegnaro M, Rojas M, Camus AM, Alexandrov K, Lang M. Expression of pulmonary cytochrome P450IA1 and carcinogen DNA adduct formation in high risk subjects for tobacco-related lung cancer. *Toxicol Lett* 1992; 64-65 Spec No: 477-483.