CYP and GST polymorphisms and survival in advanced non-small cell lung cancer patients

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Several polymorphisms in cytochrome P-450s (CYP)s and Glutathione S-transferases (GST)s have been reported to be associated with survival rates of patients with non-small cell lung cancer (NSCLC) but the studies in this regard are scarce and the results are contradictory. In this study, CYP1A1 (Ile462Val), CYP1B1 (Asn453Ser), GSTM1, GSTP1 exon 5 (Ile105Val) and exon 6 (Ala114Val) and GSTT1 polymorphisms were determined in 138 patients with advanced NSCLC to evaluate their role in survival. Of the studied CYP and GST polymorphisms only GSTP1 exon 6 variant significantly altered (improved) the survival compared to wild type \( (p=0.036) \) with median survival of 22.2 months and 16.1 months, respectively. Multivariate analysis also revealed a significant reduction of adjusted hazard ratio of death associated only with the GSTP1 exon 6 variant genotype of 0.45 (95% confidence interval (95% CI), 0.23-0.89, \( p=0.022 \)). These results show that the GSTP1 exon 6 variant genotype is associated with improved survival in the patients with advanced NSCLC.

Keywords: Cytochrome P-450, glutathione S-transferase, non small cell lung cancer, polymorphism, survival

Lung cancer is an increasing worldwide public health problem particularly in men and is responsible for the majority of the deaths arising from cancers [1, 2]. Most of the lung cancers are non-small cell lung cancer (NSCLC) and response to standard platinum based chemotherapy is rather poor in these patients [3, 4]. Thus, the investigation of the reasons behind this failure of chemotherapy and thus possibly poorer survival in these patients is very important.

Cigarette smoke has been reported to cause elevated levels of carcinogen DNA-adducts which in turn form aggressive tumors by mutating and thus inactivating tumor suppressor genes (such as p53) and thereby decrease the survival rates of patients with NSCLC [5, 6]. On the other hand, it is well known that of the lung cancer patients more than 85% of males and 60% of females are cigarette smokers [7, 8]. In Turkey, 90% of the lung cancer patients are males and 10% of them are females [8].

Metabolic activation of polycyclic aromatic hydrocarbons (PAHs) in cigarette smoke to mutagenic and carcinogenic metabolites e.g. benzo(a)pyrene 7,8–9,10 diol epoxide (BaPDE) are mediated by CYP1A1 and CYP1B1 which are phase I enzymes [9, 10]. In addition, these CYPs play a role in the metabolism of a number of chemotherapeutic agents and thus involve drug resistance [11]. The expressions of these CYPs have also been found to be higher in lung cancer patients than controls [9, 12].

These CYP genes have been found to be polymorphic. The most common alleles and polymorphisms of CYP1A1 gene are CYP1A1*2A (MspI) and CYP1A1*2C (Ile462Val) and the variant alleles have higher enzyme activities [13, 14]. Positive associations have been observed between these polymorphisms and BaPDE-DNA adduct levels in lungs of smokers or increase in lung cancer risk in various populations [9, 15, 16]. The important alleles and polymorphisms of CYP1B1 gene are CYP1B2*2 (Arg48Glyc and Ala119Ser) CYP1B*3 (Leu432Ser) and CYP1B1*4 (Asn453Ser) and the variant alleles are likely to alter the enzyme activity although this is still not conclusive [17–19]. The epidemiological studies of a possible role of CYP1B1 polymorphism in lung cancer have been reported during the recent years [20–22].

On the other hand, glutathione S-transferases (GST)s are Phase II metabolism enzymes that are involved in inactivation of mutagenic and carcinogenic DNA-reactive molecules including BaPDE [23]. GSTs also play an important role in
chemo-resistance by decreasing the cytotoxic impact of various chemotherapeutic agents in various cancers including lung [24, 25]. Various human cytosolic GST subclasses have been classified according to their genetic and biochemical properties, including GSTA, GSTM, GSTP and GSTT [26].

Since GSTs catalyze several chemotherapeutic agents and also by-products of reactive oxygen species which damage the DNA lung cancer patients may differ in response to therapy, depending on GST activity. Certain polymorphisms in GSTs are associated with changes or loss in enzyme activity. For example deletion of GSTM1 and GSTT1 genes result in loss of the corresponding enzyme activities whereas single nucleotide polymorphisms (SNPs) in GSTP1 exon 5 (Ile105Val) and GSTP1 exon 6 (Ala114Val) diminish GSTP1 enzyme activity [27, 28]. In addition, susceptible GST genotypes have been reported to be associated with high mutation frequencies in p53 and K-ras genes which may lead to more aggressive tumor phenotypes and poorer survival [6, 29, 30].

Thus, the relationship between the xenobiotic/drug metabolizing enzyme gene polymorphisms and survival in cancer patients is currently a major area of research. However, rather few molecular epidemiological studies to date have considered the role of CYP1, GSTM1, GSTP1 and GSTT1 polymorphisms in determining the survival in lung cancer. Only two studies exist with respect to CYP1A1 polymorphism and survival in lung cancer and their results are contradictory [5, 31]. Goto et al. [5] found that CYP1A1 Msp1 gene variant allele significantly shortened the survival compared to those of wild type genotypes in advanced stage NSCLC patients. This group of investigators also observed a 4.5 fold higher risk of having a mutation of the p53 gene in NSCLC patients with the CYP1A1 Msp1 variant allele than those with the wild type genotype [6]. However, Pryzgodzki et al. [31] did not observe association between the CYP1A1 (Ile462Val) polymorphism and survival rate in NSCLC patients although higher p53 mutations were noted in NSCLC patients with CYP1A1 (Ile462Val) variant.

The studies which examined the relationship between GSTM1 polymorphisms and survival in lung cancer including NSCLC revealed also rather conflicting results. Several research groups did not find significant association between the GSTM1 genotype and survival in lung cancer patients [5, 31–33]. On the other hand, Ge et al. [34] observed significantly higher survival in the GSTM1 null genotypes than GSTM1 positive genotypes whereas Sweeney et al. [35] and Gonlugur et al. [36] noted significantly shorter survival in the GSTM1 null genotypes in lung cancer patients.

Recent studies have revealed no association between the GSTP1 exon 5 (Ile105Val) polymorphism and survival of patients with lung cancer including NSCLC [32, 33, 37–39]. Contradictory findings, however, have been reported with respect to the association between GSTP1 exon 6 (Ala114Val) polymorphism and survival in patients with advanced NSCLC. Lu et al. [38] reported that this mutation have a positive effect in the survival period of patients whereas Booton et al. [39] could not find such an association in advanced NSCLC patients.

Few studies examined the link between the GSTT1 polymorphism and survival in lung cancer so far and apart of one [33] showed no evidence of a survival difference by GSTT1 genotype. [32, 35, 36]. However, Sreeja et al. [33] reported that GSTT1 null genotype along with stage was significantly associated with overall shorter survival in lung cancer patients.

In addition, to our knowledge, there exist no data with respect to the association between the GSTP1 polymorphism and survival in lung cancer. 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 recently provided by Booton et al. [39] who did not show significant association between GSTP1 polymorphisms and response to chemotherapy in NSCLC.

All this kind of information is necessary and important from the point of providing the predictive and prognostic significance of these gene genotypes in patients with NSCLC which ultimately lead to the availability of the tool needed by the clinicians to individualize the therapies and accurately predict survival [40].

In this study, we aimed to determine the association between the CYP1A1 (Ile462Val) and CYP1B1 (Asn453Ser), GSTM1, GSTP1 (Ile105Val), GSTP1 (Ala114Val) or GST1 gene polymorphisms and survival in the patients with advanced (stages III and IV) NSCLC. We also investigated the possible effects of these polymorphisms on response to first-line platinum based chemotherapy in these patients.

Materials and methods

Patients. The 138 patients with the mean age of 56 ± 9 (mean ± 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. The 126 of these patients were male with the mean age of 56 ± 9 (mean ± SD; range: 34-75) and 12 of these patients were female with the mean age of 58 ± 8 (mean ± SD; range: 44-69). All patients were accrued at 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 vital status of patients was obtained from the patients' medical records. On entry into the study, each patient had a personal interview based on a questionnaire in which they were asked to provide information that included such sociodemographic variables and smoking history. Individuals who had smoked less than 100 cigarettes in their life were defined as never smokers [20, 41]; otherwise, they were defined as smokers. Former smokers were the individuals who had quit smoking at least 1 year prior to diagnosis. Each patient had
a 7 ml sample of blood drawn into coded, heparinized tubes for immediate DNA isolation. Clinical staging was defined by both clinical assessment and chest radiography with computed tomography (CT), fiber optic bronchoscopy, bone scintigraphy, liver and adrenal CT, echography or brain CT. For pathological diagnosis, needle biopsy, and/or bronchoscopic biopsy were utilized. The staging and histological classification of the lung cancers were based on the international staging system for lung cancer and a World Health Organization report [42].

**Chemotherapy.** The patients were treated only with a variety of standard platinum-based chemotherapy regimens as first-line treatments. We subgrouped the patients according to the chemotherapy regimens as platinum and etoposide or platinum and other chemotherapeutics for statistical analysis. Due to the small number of patients in each combination of treatments e.g. cisplatin and gemcitabine (n=12) in platinum and other chemotherapeutics subgroup we combined them all as one namely platinum and other chemotherapeutics subgroup for statistical analysis. Chemotherapy was comprised of one of the following regimens:

- Platinum and Etoposide treatment subgroup: Cisplatin + Etoposide (Cisplatin, 80 mg/m² i.v. on Day 1 and etoposide, 100 mg/m² i.v. on Days 1, 2 and 3) (n=87).
- Platinum and other chemotherapeutics treatment subgroup: Cisplatin + Gemcitabine (Cisplatin, 80 mg/m² i.v. on Day 1 and gemcitabine, 1250 mg/m² i.v. on Days 1 and 8) (n=12), Cisplatin + Docetaxel (Cisplatin and docetaxel 75 mg/m² i.v. on Day 1) (n=7), Cisplatin + Vinorelbine (Cisplatin, 80 mg/m² i.v. on Day 1 and vinorelbine, 30 mg/m² i.v. on Days 1 and 8) (n=9), Cisplatin + Paclitaxel (Paclitaxel, 225 mg/m² i.v. on Day 1 and cisplatin 80 mg/m² i.v. on Day 2) (n=10), Carboplatin + Paclitaxel (Carbo- platin, 5 AUC (glomerular filtration rate+ 25mg) i.v. on day 1 and paclitaxel 225 mg/m²/2 i.v. on Days 1 and 8) (n=13).
- The patients received 2 or more cycles of chemotherapy every 3 weeks as first-line chemotherapy. The effect for chemotherapy was evaluated by the World Health Organization criteria [43]. Briefly, complete response (CR) was defined as the disappearance of all known diseases, determined by at least two observations not less than 4 weeks apart. Partial response (PR) was defined as a 50% or more decrease in total tumor size of lesions, which have been measured to determine the effect of therapy by two observations not less than 4 weeks apart. An additional criterion is the absence of new lesions or progression of any lesion. Progressive disease (PD) was defined as a 25% or more increase in the size of one or more measurable lesions or the appearance of new lesions. All other responses are classified as no change (NC), defined as a 25% or less increase and/or as a 50% or less decrease in total tumor size of lesions [45]. In this study, the responder group was consisted of patients with CR and PR and the non responsive group was consisted of patients having NC in the response and PD.

**DNA isolation** The genomic DNA used for polymorphic analysis was isolated from whole blood of patients by using DNA purification kit purchased from Promega Corporation (Madison, WI, USA) following the manufacturer’s instructions. Isolated DNA was stored at -20°C until use.

**Genotyping procedure.** Genetic polymorphism analysis for the CYP1A1 was determined by polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) method described by Cascorbi et al. [14]. The CYP1A1 Ile462Val genetic polymorphism analysis was performed in 30 µL reaction mixture containing 25 pmol of each primer (sense 5’-CTG TCT CCC TCT GGT TAC AGG AAG A, antisense 5’-TTC CAC CCG TTG CAG CAG GAT AGC C) plus 200 µmol of each dNTPs, 3 µL of 10XPCR buffer (100 mM Tris-HCl pH 9.0 25 °C, 500 mM KCl), 1.6 mM MgCl₂, 100 ng DNA and 1 unit of Taq DNA polymerase. Amplification was for 35 cycles of 0.5 min at 94 °C, 0.5 min at 63 °C and 0.5 min at 72 °C. The PCR products were digested with the BsrDI restriction enzyme and separated using 3% (w/v) agarose gel electrophoresis. The wild-type genotype gave bands at 35 bp and 149 bp, the heterozygous mutant genotype at 204 bp and 149 bp and the homozygous mutant genotype at 204 bp.

CYP1B1 genetic polymorphism analysis was determined by the PCR/RFLP method described by Bailey et al. [44]. The CYP1B1 Asn453Ser genetic polymorphism analysis was performed in a 30 µL reaction mixture containing 25 pmol of each primer (sense 5’-GGT GTT TTT GTG TAC GAA CAG TGG, antisense 5’-GCC CAC TCA AAA AAT CAT CAC TCT GCT GGT CAG GTG C) plus 90 µmol of each dNTP, 3 µL of 10 X PCR buffer (100 mM Tris-HCl pH 9.0 25 °C, 500 mM KCl), 1.5 mM MgCl₂, 500 ng DNA and 1 unit of Taq DNA polymerase. Amplification was for 35 cycles of 1 min at 95 °C, 1 min at 62 °C and 1 min at 72 °C. The PCR products were digested with the Cac8I restriction enzyme and digestion products separated using 3% (w/v) agarose gel electrophoresis. The wild-type genotype gave a band at 143 bp, the heterozygous mutant genotype at 143 and 105 bp and the homozygous mutant genotype at 105 bp.

Polymorphism analysis for the GSTM1 and the GSTT1 genes was carried out simultaneously in a single assay using a multiplex PCR approach based on the method of Abdel-Rahman et al. [45]. Briefly, 1 µg of isolated DNA was amplified in a 30 µL reaction mixture containing 30 pmol of each GSTM1 primer (sense 5’-GAA CTC CCT GCT GAA AAG CTA AAG C, antisense 5’-GTT GGG CTC AAA TAT ACG GTG G) and the GSTT1 primers corresponding to the 3’ coding region of human cDNA (sense 5’-TTC CCT ACT GGT CCT CAC ATC TC, antisense 5’-TCA CCG GTG CAT GGT CAG CA). As an internal control exon 7 of the CYP1A1 gene was co-amplified using appropriate primers (sense 5’-GAA CTG CCA CCT CAG CAG GAT TTG GAA GTG C). The reaction mixture also contained 200 µmol of each dNTP, 3 µL of 10 X PCR buffer (100 mM Tris-HCl pH 9.0 25 °C, 500 mM KCl), 1.6 mM MgCl₂ and 1 unit of Taq DNA polymerase. Amplification consisted of melting at 94 °C for 5 min, followed by 35 cycles of melting at 94 °C for 2 min, annealing at 59 °C for 1 min and extension at 72 °C for 10 min. Products were separated by 3% (w/v) agarose
gel electrophoresis. The presence or absence of a band at 480 bp corresponded to the presence or absence of GSTT1 and likewise for GSTM1 at 215 bp. A 312 bp band, corresponding to the CYP1A1 gene, was always present and was used as an internal control to indicate successful PCR amplification.

GSTP1 exon 5 (Ile105Val) and 6 (Ile105Val) genetic polymorphism analysis were determined by using the PCR-RFLP method described by Park et al. [46].

The GSTP1 Ile105Val genetic polymorphism analysis was performed in a 30 µl reaction mixture containing 25 pmol of each primer (sense, 5‘- AAT ACC ATC CTG CGT CAC CT, antisense 5‘- TGA GGG CAC AAG AAG CCC CTT) plus 100 µmol of each dNTP, 3 µl of 10X PCR buffer (100 mM Tris-HCl pH 9.0 25°C, 500 mM KCl), 1.2 mM MgCl₂, 100 ng DNA and 1 unit of Taq DNA polymerase. Amplification was for one cycle of 2 min at 95°C, 40 cycles of 0.5 min at 94°C, 0.5 min at 55°C and 0.5 min at 72°C, followed by a final 10 min extension at 72°C. Products were digested with the BsmAI restriction enzyme and the digestion products separated by 3% (w/v) agarose gel electrophoresis. The wild-type genotype gave bands at 305 bp and 138 bp, the heterozygous mutant genotype at 305 bp, 222 bp and 138 bp, and the homozygous mutant genotype at 222 bp and 138 bp.

The GSTP1 Ala114Val genetic polymorphism analysis was performed in a 30 µl reaction mixture containing 25 pmol of each primer (sense, 5‘- ACA GGA TTT GGT ACT AGC CT, antisense 5‘- TGA GGG CAC AAG AAG CCC CTT) plus 100 µmol of each dNTP, 3 µl of 10X PCR buffer (100 mM Tris-HCl pH 9.0 25°C, 500 mM KCl), 1.2 mM MgCl₂, 100 ng DNA and 1 unit of Taq DNA polymerase. Amplification was for one cycle of 2 min at 95°C, 40 cycles of 0.5 min at 94°C, 0.5 min at 55°C and 0.5 min at 72°C, followed by a final 10 min extension at 72°C. Products were digested with the BstUI restriction enzyme and the digestion products separated by 3% (w/v) agarose gel electrophoresis. The wild-type genotype gave bands at 305 bp and 138 bp, the heterozygous mutant genotype at 305 bp, 222 bp and 138 bp, and the homozygous mutant genotype at 222 bp and 138 bp.

The GSTP1 Ile105Val genetic polymorphism analysis was performed by the Kaplan–Meier survival function and log-rank tests. Statistical analysis. Chi-square analysis and Fisher 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 and GST 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, gender, smoking status, chemotherapy regimen, tumor stage and tumor histology. Only 2-sided P values < 0.05 were considered as significant. SPSS software (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis.

Results

Characteristics of the 138 patients at diagnosis are provided in Table 1. In this study, among the 138 patients 42 (30%) of them responded whereas 96 (70%) did not respond to the platinum based first-line chemotherapy. When the distributions of response to chemotherapy according to patient characteristics were evaluated they were not found to be related to age, gender, tumor histology, stage at diagnosis, chemotherapy regimen or smoking status (p>0.05, data not shown).

Due to the limited number of either homozygous or heterozygous variant allele carriers of CYPs and GSTP1 exon 5 and 6 genes, we combined these genotypes for the statistical analysis throughout the study. The distributions of CYP and GST genotypes (either alone or in combination) according to patient characteristics were also evaluated and were not observed to be related to age, gender, tumor histology, stage at diagnosis or smoking status (p>0.05, data not shown).

There were no significant associations between genotypes (either alone or in combination) and responses to first line chemotherapy (data not shown). No significant associations were also 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).

Table 1. Characteristics of 138 NSCLC patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>138</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>≤50</td>
<td>40</td>
</tr>
<tr>
<td>51-60</td>
<td>48</td>
</tr>
<tr>
<td>≥61</td>
<td>50</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>126</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Squamous cell Carcinoma</td>
<td>49</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>48</td>
</tr>
<tr>
<td>Unspecified non-small cell lung cancer</td>
<td>41</td>
</tr>
<tr>
<td>Stage at diagnosis</td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td>60</td>
</tr>
<tr>
<td>Stage IV</td>
<td>78</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Platinum+Etoposide</td>
<td>87</td>
</tr>
<tr>
<td>Platinum+others</td>
<td>51</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>13</td>
</tr>
<tr>
<td>Current</td>
<td>85</td>
</tr>
<tr>
<td>Former</td>
<td>40</td>
</tr>
</tbody>
</table>

a Cisplatin+Etoposide  
b Cisplatin+Gemcitabine, Cisplatin+Docetaxel, Cisplatin+Vinorelbine, Cisplatin+Paclitaxel, Carboplatin+Paclitaxel

CYP AND GST POLYMORPHISMS IN NON-SMALL CELL LUNG CANCER 515
The Kaplan-Meier survival functions for overall survival according to CYP and GST genotypes (either alone or in combination) were analysed. In total, 59 (43 %) deaths were observed during follow-up. Significant survival was not observed in patients with CYP1A1 variant genotype (median survival of 18.1 months for Ile/Val or Val/Val genotype and 16.3 months for Ile/Ile genotype (p = 0.523) (data not shown). However, the mutant carriers of CYP1B1 gene survived remarkably shorter than the wild type carriers of the gene (median survival of 18.2 months for wild type (Asn/Asn) genotype and 13.1 months for variant (Asn/Ser or Ser/Ser) genotype but the difference was insignificant (p = 0.089) (Fig. 1A). Significant survivals were also not observed in patients with GSTM1 (median survival of 17.1 months for GSTM1 positive genotype and 18.2 months for GSTM1 null genotype, p = 0.802) and GSTT1 genotypes (median survival 17.8 months for GSTT1 positive genotype and 16.3 months for GSTT1 null genotype, p = 0.747) (data not shown). Likewise significant survival was not observed in patients with GSTP1 exon 5 variant genotype (median survival of 16.2 months for Ile/Val or Val/Val genotype and 18.3 months for Ile/Ile genotype, p = 0.817) (data not shown). The patients who had the GSTP1 exon 6 variant genotype (Ala/Val or Val/Val) had significantly better survival compared to patients who had the wild type genotype (Ala/Ala) (p = 0.036) with median survival of 22.2 months and 16.1 months, respectively (Fig. 1B). Among the genotype combinations there were no significant association between the combined genotypes and survival periods (p > 0.05, data not shown). However, some revealed remarkable altered survival periods. For example, the patients who had both variant genotypes of CYP1A1 and GSTP1 exon 5 had shorter survival (median, 11.2 months) compared to wild type genotypes (median, 17.9 months) with marginal significance (p = 0.062, data not shown). Likewise, although not significant, the variant genotypes of both CYP1B1 and GSTP1 exon 5 genes survived remarkably shorter than the wild type genotype of the both genes (median survival, 11.1 months for variant genotypes and 18.1 months for wild type genotypes, p = 0.111) (data not shown). In addition, the patients who had both variant genotypes of GSTP1 exon 5 and GSTP1 6 had longer survival (median, 24.8 months) compared to wild type genotypes (median, 18.3 months) but this was marginally significant (p = 0.088, data not shown).

Multivariate analysis also revealed no significant hazard ratio (HR) of death associated with CYP1A1 genotypes (HR, 1.46; 95% CI, 0.69-3.07, p = 0.318) (Table 2). However, the death risk of mutant carriers of CYP1B1 gene increased with marginal significance (HR, 1.66; 95% CI, 0.93-2.96, p = 0.081) (Table 2). For the GST polymorphisms, multivariate analysis also revealed a reduced adjusted hazard ratio (HR) of death associated with the GSTP1 exon 6 variant genotype of 0.45 (95% confidence interval (95% CI), 0.23-0.89, p = 0.022) but not with the GSTM1 null (HR, 0.91, 95% CI, 0.51-1.61, p = 0.742) GSTP1 exon 5 variant (HR, 1.44; 95% CI, 0.78-2.63, p = 0.241) and GSTT1 null (HR, 1.18, 95% CI, 0.61-2.26, p = 0.627) genotypes (Table 2).

The distributions of CYP and GST combined genotypes and survival of the NSCLC patients were shown in Table 3. However, due to the very limited number of patients with null and/or variant genotypes, only the genotype combinations which were available for statistical analysis were illustrated in the table. Overall multivariate analysis revealed no significant hazard ratio (HR) of death associated with the genotype combinations. However, some of the genotype combinations...
combinations showed remarkable association with hazard ratio (HR) of death. The death risk of variant genotypes of both CYP1A1 and GSTP1 exon 5 genes increased with marginal significance as compared to wild type genotypes (HR, 3.58; 95% CI, 0.98-13.14, \( p = 0.054 \)) (Table 3). Likewise, the death risk of variant genotypes of both CYP1B1 and GSTP1 exon 5 genes increased with marginal significance (HR, 2.57; 95% CI, 0.93-7.05, \( p = 0.067 \)) (Table 3). However, combined variant genotypes of GSTP1 exon 5 and GSTP1 exon 6 decreased the HR as compared to wild type genotypes but this was statistically insignificant (HR, 0.44, 95% CI, 0.12-1.69, \( P = 0.234 \)) (Table 3).

### Discussion

In this study, we demonstrated that only GSTP1 (Ala114Val) polymorphism, but not CYP1A1 (Ile462Val), CYP1B1 (Asn453Ser), GSTM1, GSTT1 or GSTP1 (Ile105Val) polymorphism, was associated with altered (improved) survival in NSCLC patients. In addition, we observed no significant association between CYP or GST polymorphisms studied and response to first-line platinum based chemotherapy in these patients.

The result of our study in regard to the relationship between CYP1A1 (Ile462Val) polymorphism and survival is in accordance with the finding of Przygodzki et al. [31] in patients with NSCLC. However, the results of these studies are different from those of Goto et al. [5] who demonstrated that the CYP1A1Msp1 gene variant allele carrier NSCLC patients had shorter survival compared to those of wild type genotypes in 98 patients with advanced stage (III and IV) NSCLC. These findings may indicate the distinct role of these mutations of CYP1A1 gene in survival of NSCLC patients.

As CYP1A1, CYP1B1 expression is up-regulated by activation of aryl hydrocarbon receptor (AhR) through binding ligands such as cigarette smoke components and both AhR and CYP1B1 are overexpressed in lung carcinomas [12]. In addition, while AhR and CYP1A1 expression was associated with smoking, overexpression of AhR and CYP1B1 was found to be associated regardless of smoking status [12]. Furthermore, in addition to the xenobiotic response element for AhR other elements involve in the regulation of CYP1B1 (such as cyclic AMP-response element and an

### Table 3. The distributions of CYP and GST combined genotypes and survival of NSCLC patients.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Overall Survival</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>HR (95% CI)(^a)</td>
</tr>
<tr>
<td>CYP1A1 Ile/Val+Val/Val + CYP1B1 Asn/Ser+Ser/Ser</td>
<td>2.16 (0.57-8.18)</td>
</tr>
<tr>
<td>CYP1A1 Ile/Val+Val/Val + GSTM1 null</td>
<td>1.02 (0.30-3.44)</td>
</tr>
<tr>
<td>CYP1A1 Ile/Val+Val/Val + GSTT1 null</td>
<td>1.56 (0.52-4.69)</td>
</tr>
<tr>
<td>CYP1B1 Asn/Ser+Ser/Ser + GSTM1 null</td>
<td>0.95 (0.34-2.60)</td>
</tr>
<tr>
<td>CYP1B1 Asn/Ser+Ser/Ser + GSTT1 null</td>
<td>1.38 (0.41-4.67)</td>
</tr>
<tr>
<td>CYP1A1 Ile/Val+Val/Val + GSTP1 exon 5 Ile/Val+Val/Val</td>
<td>3.58 (0.98-13.14)</td>
</tr>
<tr>
<td>CYP1A1 Ile/Val+Val/Val + GSTP1 exon 6 Ala/Val+Val/Val</td>
<td>0.53 (0.06-4.69)</td>
</tr>
<tr>
<td>CYP1B1 Asn/Ser+Ser/Ser + GSTP1 exon 5 Ile/Val+Val/Val</td>
<td>2.57 (0.93-7.05)</td>
</tr>
<tr>
<td>CYP1B1 Asn/Ser+Ser/Ser + GSTP1 exon 6 Ala/Val+Val/Val</td>
<td>0.83 (0.18-3.74)</td>
</tr>
<tr>
<td>GSTM1 null + GSTT1 null</td>
<td>1.06 (0.43-2.62)</td>
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<tr>
<td>GSTM1 null + GSTP1 exon 5 Ile/Val+Val/Val</td>
<td>1.43 (0.60-3.42)</td>
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<tr>
<td>GSTM1 null + GSTP1 exon 6 Ala/Val+Val/Val</td>
<td>1.62 (0.20-1.62)</td>
</tr>
<tr>
<td>GSTT1 null + GSTP1 exon 5 Ile/Val+Val/Val</td>
<td>1.53 (0.61-3.82)</td>
</tr>
<tr>
<td>GSTP1 exon 5 Ile/Val+Val/Val + GSTP1 exon 6 Ala/Val+Val/Val</td>
<td>0.44 (0.12-1.69)</td>
</tr>
</tbody>
</table>

\(^a\)HR: hazard ratio, 95% CI: 95% confidence interval. Null and/or variant genotypes compared to present and/or wild type genotypes. HR and 95% CI values were determined by using Cox proportional hazards model that was adjusted for age, gender, tumor histology, tumor stage, smoking status, chemotherapy regimen and response to chemotherapy.

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enhancer consisting of steroidogenic factor 1 elements) [47] which were not assessed for CYP1A1. Thus, overexpression of CYP1B1 gene and the increase in intracellular oxidative stress and promotion of cell growth as well as PAH activation to carcinogenic metabolites are likely to favor this CYP to be more responsible for the formation of more aggressive tumors and possibly worsening of the survival. Moreover, evidence has been presented for an association between the frequency of tobacco-induced p53 mutations and CYP1B1 polymorphism in cancer patients [48]. Hence, the polymorphisms of CYP1B1 might cause an altered function of the enzyme thereby determine inter individual differences in susceptibility to carcinogenesis and survival among cancer patients. However, association between the most common polymorphisms of CYP1B1 gene and alteration in enzyme activity is not clear [17–19]. The enzyme of the variant allele CYP1B1*4 [Asn453Ser] CYP1B1.4 has been observed to have a reduced enzyme activity in conversion of BaP to BaP –7.8 dihidrodiol in yeast [19] and by posttranslational degradation in COS-1 cells [49]. The Bandiera and co-workers [49] based on their findings have suggested that the reduced metabolic activation of exogenous and endogenous procarcinogens by CYP1B1.4 could be expected to be protective as observed in endometrial cancer [50]. However, a recent study by Sissung et al. [51] did not confirm the study of McGrath and co-workers [50] in endometrial cancer. In addition, no association between this polymorphism and various other cancers including lung has been reported in recent years [21, 22, 52–54]. These findings are not in accordance with the findings of Aklilliu et al. [19] and Bandiera et al. [49]. Moreover, CYP1B1 polymorphisms were found to be not significantly associated with the survival in patients with NSCLC in this study. Even a notable trend towards worsening of the survival in the CYP1B1 mutant allele carriers of advanced NSCLC patients was observed.

The lack of association observed between GSTM1 polymorphism and survival in NSCLC patients in the current study is in line with some of the previous studies [5, 31–33] but in contrast to others [34–36]. Although the picture appears to be rather complicated, the current value of evidence seems to be in favor of lack of relationship between the GSTM1 polymorphism and survival in lung cancer patients.

This and previous studies carried out in regard to GSTT1 [32, 35, 36] or GSTP1 (Ile105Val) [32, 33, 35, 38, 39] polymorphism and survival did not reveal any association in lung cancer patients including NSCLC. Thus, the overall lack of association between GSTT1 or GSTP1 exon 5 genotype and survival in lung cancer patients appears to be a consistent finding.

Our observations concerning the protective effect of GSTP1 (Ala114Val) polymorphism on the survival of patients with advanced NSCLC is in line with those of Lu et al. [38] whereas in contrast to those of Booton et al. [39]. The lack of protective association observed by Booton et al. [39] might be due to their short patient follow-up period (about 2 years) since the GST genotype effect on survival is likely to become apparent after 3 years and more follow-up [5, 35, 38].

On the other hand, no association was observed in our study between the CYP or GST genotypes and response to first-line chemotherapy. Moreover, the effects of CYP and GST polymorphisms on response were not observed to vary among the patients treated either with platinum and etoposide or platinum and other chemotherapeutics. These findings are likely to show that the studied CYP and GST polymorphisms are not functioning as a predictor of response to these two distinct platinum-based chemotherapy regimens, consisting of drugs among which namely cisplatin, carboplatin, etoposide and docetaxel are known substrates of especially GSTP1 enzyme [24, 55, 56], in advanced NSCLC patients. Our results are in line with those of Booton et al. [39] who recently observed no association between the GSTP1 polymorphisms and platinum based treatment in advanced NSCLC patients [39]. Regarding CYP1A1, CYP1B1, GSTM1 and GSTT1 polymorphisms and response to treatment, no other information is available in NSCLC patients. Nevertheless, in a recent study in another cancer namely ovarian cancer patients Beeghly et al. [57] observed no association between GSTM1 and GSTT1 polymorphisms and response to overall platinum based treatment or the chemotherapeutic agent subgroups.

Recent studies have shown that the simultaneous analysis of polymorphisms of xenobiotic/drug metabolizing enzyme genes may correlate with clinical outcome better than the single polymorphism studies due to the fact that they share overlapping substrate specificities and/or further enhancement or reduction of their activities towards the substrates. For example the combined GSTM1 null and GSTP1 exon 5 variant genotype was associated with better survival but not response to treatment in ovarian cancer patients [57]. Similar findings were reported for brain tumors for the same gene interactions [60]. In the current study, the combined CYP and GST genotypes were not found to be associated with response in NSCLC patients. However, patients carrying the combined CYP1A1 and GSTP1 exon 5 variant alleles or CYB1 and GSTP1 exon 5 variant alleles demonstrated notable trends toward shorter survival. On the other hand, survival advantage was seen in the patients with combined GSTP1 exon 5 and GSTP1 exon 6 variant alleles.

At this stage the mechanisms underlying the favorable survival outcome associated with GSTP1 exon 6 variant allele are not clear and should be further investigated.

In summary, this study shows that the GSTP1 exon 6 variant genotype is associated with improved survival in the patients with advanced NSCLC.

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References


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