Glutathione S-transferase M1 and T1, CYP1A2-2467T/delT polymorphisms and non small-cell lung cancer risk in Tunisian sample

B’chir Fatma a,b,*, Taieb Aida b, J. Arnaud Maurice c, Saad Saguem b

a Laboratory of Natural Substances, National Institute of Research and Physical–Chemical Analysis (INRAP), Technopole Sidi Thabet 2020, Tunisia
b Metabolic Biophysics and Applied Pharmacology Laboratory, Department of Biophysics, Medicine Faculty of Sousse, 4002 Sousse, Tunisia
c Nutrition and Biochemistry, 1814 La Tour de Peilz, Switzerland

Received 20 February 2012; accepted 7 May 2012
Available online 16 June 2012

KEYWORDS
GSTT1; GSTTM1; CYP1A2; Polymorphisms; Lung cancer

Abstract The present study investigated the impact of metabolic gene polymorphisms in modulating lung cancer risk susceptibility. Gene polymorphisms encoding Cytochrome 1A2 (CYP1A2) and Glutathione-S-transferases (GSTT1 and GSTM1) are involved in the bioactivation and detoxification of tobacco carcinogens and may therefore affect lung cancer risk.

In order to assess association between lung cancer and GSTT1, GSTM1 and CYP1A2-2467T/delT, variant allele (CYP1A2*1D) polymorphisms, a case-control study of healthy and smoking lung cancer patients in Tunisian population was conducted. Polymorphisms of GSTs were assayed by multiplex PCR. Genotype polymorphism of CYP1A2*1D was determined by PCR-RFLP assay. Odds Ratio was used for analysing results.

There was no association with any increase of lung cancer risk in GSTT1 null genotype [OR: 0.83 (0.47–1.45)] as well as in GSTM1 null genotype [OR: 0.78 (0.37–1.62)]. However, a significant association between lung cancer and the homozygous mutate variant of the CYP1A2 [(OR = 4.7 (1.55–29.78)] was observed.

* Corresponding author at: Laboratory of Natural Substances, National Institute of Research and Physical–Chemical Analysis (INRAP), Technopole Sidi Thabet 2020, Tunisia. Tel.: +216 97 327 107; fax: +216 73 224 899.
E-mail address: bchirfatma@hotmail.fr (B. Fatma).
Peer review under responsibility of Ain Shams University.
1. Introduction

Lung cancer is the most common cause of death in several developing countries. Its increasing incidence and mortality are observed all over the world [1]. Tobacco smoking is the main and predominant risk factor for lung cancer disease. Worldwide, the lung cancer mortality in the population due to smoking is estimated to 79% for men and 48% for women [2,3]. However, an increased incidence of lung cancer has also been observed in non-smokers [3,4] and in addition to tobacco smoke, lung cancer is known to be associated with other risk factors such as diet, air pollution and occupational exposures to carcinogens [3].

Although smoking remains the most known risk factor for lung cancer development, it might be hypothesised that early-onset of lung cancer among both smokers and non-smokers may have a stronger genetic component in its pathology [5,6]. This genetic component may modulate the risk associated with environmental factors.

Polymorphisms in genes encoding for xenobiotic metabolism of phase I and phase II enzymes such as the cytochrome P450 1A2 (CYP1A2) phase I enzyme and the glutathione-S-transferase theta 1 gene (GSTT1) and the glutathione-S-transferase mu 1 gene (GSTM1) isoenzymes from phase II detoxifying family are more studied recent years in particular in the field of their association with diseases.

GSTs catalyse electrophilic metabolites through conjugation with reduced glutathione followed by excretion from the body. Both GSTT1 and GSTM1 are particularly attractive candidates for lung cancer susceptibility because of their involvement in the detoxification of several carcinogenic compounds found in tobacco smoke and in chemicals and environmental exposure [7].

GSTM1 has been found to detoxify Polycyclic Aromatic Hydrocarbons (PAH) present in tobacco smoke. GSTT1 is involved in the metabolism of smaller compounds also found in tobacco smoke, such as monohalomethane and ethylene oxide [8]. So deletions in GSTs genes are considered as potential modifiers of individual lung cancer risk.

CYP1A2 is a phase I enzyme, involved in the activation of several indirect carcinogens such as nitrosamines, Aromatic Heterocyclic Amine (AH)) which are present in the mainstream of tobacco smoke [9]. As a consequence, the formation of reactive metabolites and their binding to DNA give then stable adducts which are considered as the critical step in the initiation of carcinogenic process. Thus, high activity of CYP1A2 was therefore suggested as a potential risk factor for lung cancer.

Several environmental factors as well as tobacco smoke have been shown to induce CYP1A2 activity. As the study of Pavanello [10,11], it was shown that genetic polymorphism in non-coding CYP1A2 promoter, the CYP1A2*1D variant allele which consists of the deletion of T base (−2467T/delT) increases the CYP1A2 activity in smokers. This information suggests that this mutation may interfere and affect the susceptibility to lung cancer.

In this context, this study has been undertaken to address the association of both metabolic genes CYP1A2*1D phase I enzyme and GSTT1-M1 phase II enzymes with lung cancer susceptibility among Tunisian smokers.

2. Subjects and methods

Ninety-eight healthy subjects and 101 lung cancer cases, including 41 patients with Adenocarcinoma (AD) and 60 patients with Squamous Cell Carcinoma (SCC) histological cell types participated to this study.

Lung cancer cases were enrolled from “CHU Farhat Hached” central hospital of Tunisia from June 2004 to July 2005. They are all men and current smokers. Classifications and histological types of lung cancer cases were assessed by fibre optic bronchoscopy technique.

At recruitment, each participant was interviewed to obtain detailed information on their tobacco use and possible exposure to carcinogens through dietary habits and professional activities. All information regarding participants was made anonymous after collection of data and blood samples. Informed consent was obtained from each participant before interview and blood collection for genetic analysis.

The study was approved by the Ethics Committee of the Hospital in Sousse [12].

Variables selected from the data are: age, histology of cell cancer, smoking status, packs-years of smoking, types of cigarette smoked and the mean daily cigarettes smoked.

Other variables, such occupational exposure, family history of cancer, were available but not considered in this analysis.

The Controls selected were all men and current smokers. The controls were matched for gender, age and smoking status with the cases.

A 5 ml sample of whole blood was collected from each subject in a 7 ml K3ETDA tube for genetic analysis.

The presence of GSTT1 and GSTM1 genes was determined using multiplex Polymerase Chain Reaction (PCR) methodology.

Allelic variants −2467 T/delT (allele* 1D) of CYP1A2 gene were performed by PCR-restriction fragment length polymorphism analysis [11].

3. Statistical analysis

Data were analysed using SPSS 0.11 statistical software. Odds Ratio (OR) was calculated by logistic regression in exact 95% confidence interval (CI).

4. Results

Relevant characteristics of patients and controls are given in Table 1. Cases were ever smokers more than controls. The
mean value of pack-years was higher among patients than controls ($P < 0.05$).

Table 2 gives the analysis and the distribution of GSTT1, GSTM1 and CYP1A2*1D genotypes among cases and controls. GSTM1 null genotype was more frequent than GSTT1 null genotype. They were present respectively in 15.8% and in 45.9% among healthy controls.

The prevalence of GSTT1 null genotype in lung cancer patients was 19.4% compared to 15.8% in controls. Frequencies of GSTM1 gene deletion were 49.5% in patients and 45.9% in healthy subjects. The two samples did not differ significantly in frequencies of null genotypes between patients and controls.

Both GSTT1 and GSTM1 gene deletions were not associated with any lung cancer risk. Their odds Ratios (ORs) are 0.83 (0.47–1.45 (CI)) for GSTT1 and 0.78 (0.37–1.62(CI)) for GSTM1.

For CYP1A2*1D (−2467deltT) distribution, the heterozygous and homozygous mutated variants frequency was present in 11% of controls and 24% of lung cancer cases.

There is a significant association between lung cancer and the homozygous mutated variant of the CYP1A2 [OR = 4.7 (1.55–29.78)]. Subjects with mutated homozygous CYP1A2 delt/delt genotype present an increased risk of lung cancer of fourfold higher than subjects with wild type genotype.

Considering the cell type lung cancer, the genotype −2467delt/delt frequency was 7% in AD lung cancer patients and totally absent in SCC.

For GSTT1 null genotype, it was more frequent in SCC than AD (21.6% versus 4.87%), while the GSTM1 null genotype distribution was almost the same in AD and in SCC (Table 3). A significant association between GSTT1 null genotype and AD lung cancer risk was registered [OR = 2.35(1.13–5.66)].

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>101 (100)</td>
<td>60 (100)</td>
<td>41 (100)</td>
<td>98 (100)</td>
</tr>
<tr>
<td>Female</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>56</td>
<td>56</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>Range</td>
<td>37–84</td>
<td>38–82</td>
<td>37–84</td>
<td>36–84</td>
</tr>
<tr>
<td><strong>Smoking by pack-years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>45</td>
<td>45</td>
<td>40</td>
<td>38</td>
</tr>
<tr>
<td>Range</td>
<td>13–120</td>
<td>23–114</td>
<td>23–120</td>
<td>15–114</td>
</tr>
<tr>
<td><strong>Years smoked</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Range</td>
<td>15–59</td>
<td>17–59</td>
<td>15–50</td>
<td>10–64</td>
</tr>
<tr>
<td><strong>Mean daily cigarettes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Range</td>
<td>10–65</td>
<td>10–60</td>
<td>20–65</td>
<td>10–50</td>
</tr>
</tbody>
</table>
enhanced risk of lung cancer [22–25]. The CYP1A2 enzyme is highly expressed in liver and liver also expressed at lower frequencies in different parts of lungs in humans [16–18]. The CYP1A2 plays an important role in the bioactivation of precarcinogenic environmental toxins [19–21] and it may be implicated in the initiation and the development of lung cancer.

Therefore the level of expressions and catalytic activities of CYP1A2, GSTT1 and GSTM1 enzymes and their metabolic balance, may be an important determining factor underlying lung cancer susceptibility.

Several studies have suggested that GSTs genotypes may play mainly role in determining susceptibility to lung cancer because of their faculty to eliminate carcinogenic electrophilic compounds. They have reported that individuals polymorphic in carcinogen-detoxifying genes may have a weak ability to eliminate these compounds. The lack of these phase II enzymes leads to the decrease of detoxification pathway and accumulation of carcinogenic metabolites.

The most common polymorphism in GSTT1 and GSTM1 consists of a deletion of the whole T1 and M1 genes, resulting in the lack of active enzymes. The lack of these phase II enzymes leads to the decrease of detoxification pathway and accumulation of carcinogenic metabolites.

In this study we have examined the impact of the GSTT1, GSTM1 and CYP1A2 genes polymorphism on lung cancer susceptibility among Tunisian population.

GSTT1, GSTM1 and CYP1A2 were the most extensively investigated polymorphic gene enzymes for lung cancer risk because of their potential involvement in carcinogenesis process [8,13–15]. So the polymorphism in the metabolic activities of these enzymes may be an important determinant factor for lung cancer risk.

The most common polymorphism in GSTT1 and GSTM1 consists of a deletion of the whole T1 and M1 genes, resulting in the lack of active enzymes. The lack of these phase II enzymes leads to the decrease of detoxification pathway and accumulation of carcinogenic metabolites.

The CYP1A2 enzyme is highly expressed in liver and liver also expressed at lower frequencies in different parts of lungs in humans [16–18]. The CYP1A2 plays an important role in the bioactivation of precarcinogenic environmental toxins [19–21] and it may be implicated in the initiation and the development of lung cancer.

Therefore the level of expressions and catalytic activities of CYP1A2, GSTT1 and GSTM1 enzymes and their metabolic balance, may be an important determining factor underlying lung cancer susceptibility.

Several studies have suggested that GSTs genotypes may play mainly role in determining susceptibility to lung cancer because of their faculty to eliminate carcinogenic electrophilic compounds. They have reported that individuals polymorphic in carcinogen-detoxifying genes may have a weak ability to eliminate these compounds. The lack of these phase II enzymes leads to the decrease of detoxification pathway and accumulation of carcinogenic metabolites.

However data are often in conflict because of the variations in the GSTT1 and GSTM1 null allele frequencies in different ethnic groups [26,27].

In fact, inter-ethnic variations in GSTT1 and GSTM1 null genotype frequencies are obviously recorded. 50% of Caucasians population present a GSTM1 null genotype [28], which is in accordance with our Tunisian population (45.9%), 33%, of African American and 45% of Japanese have deletion in the GSTM1 gene [29].

Relationship between GSTM1 null genotype and lung cancer risk is in conflict according to the results reported from various researcher groups, some studies showed an increased lung cancer risk for GSTM1 [22,33] deleted gene and other studies did not mentioned any association [25,30,31].

In our study, the GSTM1 null genotype was not associated with any increased risk of lung cancer.

For GSTT1 null genotype, the Tunisian population frequencies were so different to that of frequencies reported worldwide by Nelson et al. [27]. Our results showed a very low GSTT1 null genotype frequency (15.8% in healthy subjects) compared to other different ethnic groups. Deletion of GSTT1 loci was present in 64% of Chinese, 60% of Koreans, 28% of Caucasians and 22% of African American [27].

Despite of the low frequency of GSTT1 gene deletion in the Tunisian population of this study, an association by histological type cells was observed while an absence of association with global lung cancer risk was recorded. Therefore our results are not in accordance with those of Sreeja and Squiren [14,32,33] showing that lung cancer patients who were smokers and having a GSTT1 null genotype had an increased risk [OR = 2.24, 95%CI (1.020–7.968)] for lung cancer compared with non-smokers.

However this result suggests that individuals with GSTT1 null genotype, in particular the smokers, are more at risk to develop AD lung cancer and not SCC.

The deletion of GSTT1 loci and the consequent lower detoxification of xenobiotics, particularly the toxins found in tobacco smoke and environmental pollution, suggest that individuals with deleted GSTT1 gene were more susceptible to disease induced by toxic substances present in environment and subsequently may contribute to AD lung cancer.

The development of AD cells form occurs in the peripheral region of the lung, the area where stored the electrophilic metabolites of main stream smoke [34].

We can suggest that the presence of GSTT1 loci and its detoxifying functional enzymes may play a defensive role against the development of AD lung cancer. The genetic polymorphism of GST probably plays a role in the pathogenesis of lung cancer. However, the polymorphisms of these genes may also interact with dietary intake of isothiocyanates provided by cruciferous vegetables in the process of lung cancer. Isothiocyanates that are metabolised by GST enzymes have been shown to reduce lung cancer risk and this reduction in risk was stronger among persons with GSTM1 null genotype who metabolise and eliminate these compounds less rapidly [25].

We observed a higher prevalence of CYP1A2*1D gene polymorphisms (t/delt, del/delt) in lung cancer patients when compared to controls. A strong and significant association was found between a mutated variant homoyzous form CYP1A2 delt/delt and lung cancer risk [OR = 4.07 (1.55–29.78)]. Individuals with delt/delt allele variant present a fourfold higher risk to develop lung cancer. This observation, therefore suggests that this mutated genotype may play an important role in the development of lung cancer. However, the analysis including both CYP1A2 heterozygous and homoyzous variants show no significant association with histological lung cancer cell types risk [OR = 2.08 (0.94–4.71)], although the CYP1A2 homozygous variant frequency was higher in patient with AD lung cancer histological type.
As shown, no correlation was observed between GSTM1 null genotype, CYP1A2 mutated variants and lung cancer cell types. This observation may be supported by the small size samples presented in this study which may explain our inability to identify a significant association of GSTM1 null genotype and CYP1A2*1D with lung cancer cell types susceptibility.

6. Conclusion

From the present study, the results showed that only CYP1A2 homozoygous mutated genotype was strongly associated with the risk of lung cancer. This association appears to be potentially relevant in our population in contrast with GST genes.

These results also show that phase II metabolic enzymes do not seem to be involved in lung cancer risk despite its preventive role.

According to the association between these three gene polymorphisms and lung cancer cell types, our study indicates that only GSTT1 genotype was associated with AD lung cancer development.

Certain limitations should be mentioned in this study. Firstly the results should be viewed with caution due to the small number of subjects genotyped in this study.

Secondly, it would be more efficient to study lung cancer risks combining the effects of the three polymorphisms CYP1A2*1D, GSTT1 and GSTM1, even if the combined action of phase I and phase II enzymes with increased activation and decreased detoxification of tobacco smoke carcinogenesis has been hypothesised to lead to increased lung cancer risk. Individuals having defective for more than one of these genes are at greater risk for lung cancer than those having a defective genotype of only one gene.

Sreeja et al. study has investigated the combination effects of CYP1A1, GSTT1 and GTTM1 genotypes with lung cancer susceptibility and has indicated that the CYP1A1 homozygous variant genotype and GSTT1 null genotype are more strongly associated with lung cancer risk [14]. It was not possible to apply this combined effect test in our study due to the small population recruited. However, it would be useful to study the combining effects of the majority of phase I and phase II metabolic genotypes. The presence of CYP1A2 or/and CYP1A1 variant proteins may result in increased formation of carcinogenic metabolites due to the enhanced phase I enzyme activity, with the detoxification of the reactive metabolites being reduced by the absence of functional phase II enzymes like GSTT1 and GSTM1, which might have a role in the initiation of lung cancer.

So it seems useful to propose for further investigations to study genotypes of the combining effects of both CYP1A2 and CYP1A1 phase I enzymes and GSTT1 and GSTT1 phase II enzymes in people exposed to environmental toxins. This way will be more helpful and coherent for the assessment and to define lung cancer risk.

Acknowledgments

We thank all the staff of the Biophysics Department for their help and assistance.

References


