Cancer Epidemiology 39 (2015) 947–955

Contents lists available at ScienceDirect

Cancer Epidemiology

The International Journal of Cancer Epidemiology, Detection, and Prevention

journal homepage: www.canceepidemiology.net

Genetic polymorphisms in *GSTM1*,*GSTT1* and *GSTP1* genes and risk of lung cancer in a North Indian population

Neha Sharma[a], Amrita Singh[a], Navneet Singh[b], Digamber Behera[b], Siddharth Sharma[a,*]

[a] Department of Biotechnology, Thapar University, Patiala, Punjab 147002, India
[b] Department of Pulmonary Medicine, Post Graduate Institute of Education and Medical Research (PGIMER), Sector 14, Chandigarh, India

ARTICLE INFO

Article history:
Received 3 March 2015
Received in revised form 9 September 2015
Accepted 14 October 2015
Available online xxx

Keywords:
@ GSTM1
GSTT1
GSTP1
Polymorphism
Lung cancer
Genotype

ABSTRACT

Background: A number of studies done so far in different populations have shown that polymorphisms within the *GST* genes play an important role in determining individual susceptibility to lung cancer; however, data obtained so far have been contradictory within the same or different populations. Few studies have focused on the combinatorial effect of the *GST* genes on susceptibility to lung cancer and also for different histological subtypes. Our aim is to investigate the roles of *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms as genetic modifiers of risk for lung cancer and histological subtypes using a larger sample size in a North Indian population.

Methods: In total 540 subjects (270 lung cancer cases and 270 controls) were evaluated for the *GST* polymorphism. Genotyping for the *GSTM1*, *GSTT1* and *GSTP1* gene was done by using a multiplex PCR and PCR-RFLP method.

Results: GSTM1 null genotype was found to be associated with lung cancer (OR = 1.65, 95%CI: 116–2.3, $P = 0.005$) and this risk was higher in cases of adenocarcinoma (ADCC). *GSTT1* and *GSTP1* did not show any significant association with lung cancer; however, when stratified for histological subtypes a significant association was observed for ADCC and small-cell lung cancer (SCLC) for both *GSTT1* null and variant *GSTP1* genotypes. The combined 'at risk' genotypes of null *GSTM1* and *GSTT1* genes were found to be associated with lung cancer risk, and this risk was higher in cases of ADCC (OR = 4.09, 95%CI: 110–10.2, $P = 0.002$). There is a twofold increased risk for lung cancer with the null *GSTM1* and wild-type *GSTP1* genotypes ($P = 0.0004$); similarly, a fourfold increased risk was observed with the null *GSTT1* and wild-type *GSTP1* genotypes ($P = 0.0001$).

Conclusions: The deficient *GST* genotypes seem thus to be important risk modifiers for lung cancer and related histological subtypes, especially in combination.

1. Introduction

In recent years lung cancer (LC) has emerged as one of the leading known cancers, accounting for 13% of all cancers; in India it is the most frequent cancer occurring among men [1]. Tobacco smoking is a well-known risk factor for lung cancer, and 90% of lung cancers are attributed to smoking. Tobacco smoke is known to contain a plethora of chemicals, among which more than 50% are carcinogenic. Some known carcinogens found in tobacco smoke are PAH (polycyclic aromatic hydrocarbons), NNK 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone, nitroso-compounds, aromatic amines etc. [2].

An inbuilt xenobiotic metabolic system helps to get rid of the carcinogens present in tobacco smoke once inhaled. The carcinogens are processed sequentially: first activation of the pro-carcinogens occur by phase I enzymes which are predominately mediated by cytochrome-P450s; these are reported to catalyze many C-, N-, and S- oxidation and de-alkylation reactions. A number of CYPs have been identified to date, but the ones common in xenobiotic metabolism are *CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2E1* and *CYP3A4* [3]. The activated carcinogens subsequently bind DNA and form conjugates, resulting in DNA adducts which may eventually produce somatic mutations and trigger carcinogenesis. However, these DNA adducts can be eliminated from the cells with the help of glutathione-S-transferases (GSTs) [4]. GSTs are a large family of enzymes, constituting a phase II detoxification system along with *N*-acetyltransferase and sulfotransferase. GSTs

Abbreviations: LC, lung cancer; SQCC, squamous cell carcinoma; ADCC, adenocarcinoma; SCLC, small cell lung carcinoma; GSTs, glutathione-S-transferases; OR, odds ratio; 95% CI, 95% confidence interval; PY, pack years; SD, standard deviation; ETS, environmental tobacco smoke.

* Corresponding author.
E-mail address: siddharthsharma.phd@thapar.edu (S. Sharma).

http://dx.doi.org/10.1016/j.canep.2015.10.014
1877-7821/

play a vital role in modulating the induction of other enzymes and proteins for cellular functions – for example, DNA repair – and are therefore important in maintaining genomic integrity. Human GSTs are further classified as alpha, mu, pi and theta [4]. All act on different substrates, converting them into soluble compounds and thus facilitating their excretion. The GSTs are polymorphic in nature, and the enzyme levels expressed depend upon their induction and genetic polymorphism [5]. Inter-individual differences in susceptibility towards carcinogenesis and hence cancer depend upon the variation in the metabolism of pro-carcinogens and carcinogens, which is directly influenced by genetic variations in the xenobiotic-metabolizing enzymes. Thus, the genetic polymorphisms can be investigated as potential risk modifiers. Abundant polymorphisms are known in the GST family, and the three classes (*GSTM1, GSTT1*, and *GSTP1*) of GST are defined as having well characterized polymorphisms [6]. *GSTM1* is responsible for elimination of extensive carcinogens, certain reactive oxygen species (ROSs) and many chemotherapeutic agents, whereas *GSTT1* also has a profound role in the removal of environmental carcinogens such as 1,3-butadiene and ethylene oxide in smoke [7] (Fig. 1).

GSTM1 and *GSTT1* gene deletions in the population render the enzymes inactive, thereby hindering the detoxification mechanism. The prevalence of *GSTM1* null genotype varies in different populations; for example, it is 38% in Caucasians and 67% in those of Japanese ethnicity [8,9]. The *GSTM1* null genotype has been associated with increased risk for many cancers, including lung, liver head and neck, colon, bladder, skin and pancreatic cancers. Various studies illustrate varied associations between GSTM1 gene deletion and lung cancer risk. Some studies have suggested such an association in e.g. an Asian population [10]; on the other hand some studies have confirmed only a weak association between GSTM1 and lung cancer [11]. In line with the above statement, no association is reported with overall lung cancer in Turkish and Japanese populations [12–14]. There is mounting evidence that *GSTT1* deletion would alter the xenobiotic-metabolizing capacity and hence increase the susceptibility to carcinogenesis [5]. In the Asian population, the frequency of the *GSTT1* null genotype is higher than that in other populations [15]. For example, some studies found that the *GSTT1* null genotype was associated with an increased risk for lung cancer in an Asian population, while have others reported negative results [16].

GSTP1 is the major GST isoenzyme which is found to be expressed in the lungs and has been shown to be overexpressed in malignant tissue as compared to normal tissues [17]. A single nucleotide polymorphism (SNP) (rs1695) found in exon 5, which is due to an $A^{313}G$ substitution, results in the change of isoleucine to valine ($Ile^{105}Val$) and hence leads to reduced *GSTP1* enzyme activity. Therefore, individuals with reduced or no GST enzymatic activity may be at a greater risk for cancer due to decreased detoxification of carcinogenic and mutagenic compounds. The *GSTP1 313G* variant has been extensively studied and occurs at frequencies of 14–20% among Africans, 28–32% among Caucasians and 14–18% among Asians [18]. A recent pooled analysis has failed to show any association between *GSTP1* $Ile^{105}val$ polymorphism and overall risk for lung cancer. However, when stratified according to ethnicity, a positive association was observed only in the case of an Asian population [19].

The literature described above indicates that genetic variants in the GSTs might be accountable for lung cancers. Studies done in the Indian subcontinent have shown conflicting results, and apart from issues related to sample size, some studies have not evaluated the role of *GST* polymorphisms in relation to histological subtypes or the role of gene–gene and gene–environment interactions [20–23]. Therefore, the aim of this study was to further investigate the role of *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms as genetic modifiers of risk for lung cancer, for histological subtypes, and their relationship with tobacco smoking using a larger sample size in an Indian population. We performed association analysis in this case-control study for the three genetic variants of the *GST* gene individually and also in different combinations in order to evaluate the cumulative role of these in modifying the susceptibility towards lung cancer (Fig. 2).

2. Material and methods

2.1. Study subjects

A total of 270 lung cancer patients were recruited for this study from the Department of Pulmonary Medicine, Post Graduate Institute of Medical Education and Research (PGIMER) Chandigarh, India. This study was reviewed and approved by the institute ethics committee of PGIMER. Informed written consent was obtained from all participants or their representatives. In brief, eligible cases included all patients who were newly diagnosed with primary lung cancer. All the recruited patients were histopathologically diagnosed as having non-small-cell lung cancer (NSCLC) or small-cell

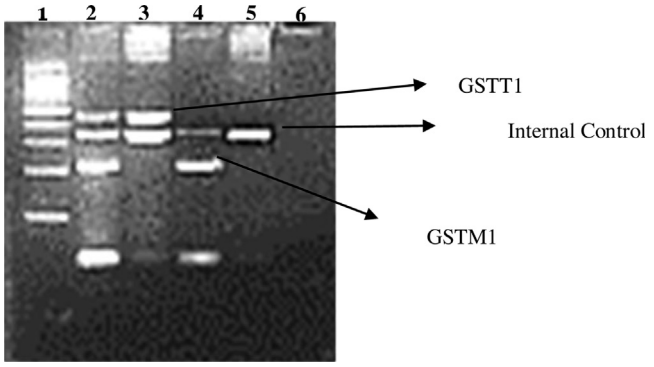

Fig. 1. Multiplex PCR. analysis of the GST polymorphisms. The PCR reaction was performed as described in the text. The resulting DNA fragments were separated by electrophoresis on a 1.5% agarose gel containing ethidium and were subsequently visualized by UV detection. The analysis resulted in the unequivocal assignment of the following genotypes: Lane-1: is 100 bp DNA ladder. Lane-2: show GSTT1 positive genotype (480 bp) and GSTM1 positive genotype (215 bp) and a band at 350 bp corresponding to internal control (Albumin gene). Lane-3: shows GSTT1 positive genotype (480 bp) with null allele for GSTM1 gene. Lane-4: shows GSTM1 positive genotype (215 bp) with null allele for GSTT1genes. Lane-5: shows individuals with null allele for both GSTM1 and GSTT1 genes. Lane-6: negative control.

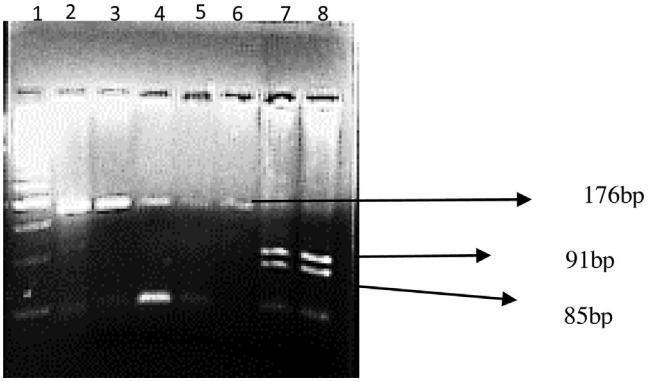

Fig. 2. Agarose gel of the GSTP1 PCR products after digestion with Alw26I for the detection of GSTP1-105 polymorphisms. Lane: 2-6: Homozygous for the wild-type GSTP1 gene (GSTP1 Ile/Ile); Lane 7–8: Homozygous for the mutated GSTP1 gene (GSTP1 Val/Val).