GENETIC POLYMORPHISMS AND THE RISK OF LUNG CANCER IN TUNNEL WORKERS IN RIO DE JANEIRO, BRAZIL

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ABSTRACT

Individual susceptibility to chemically induced cancer may be partly explained by genetic differences in the activation and detoxification of procarcinogens. Numerous polymorphisms of metabolism genes have been identified and their role in individual susceptibility to cancer has been observed. Many studies have shown that variant *CYP1A1* alleles combined with *GSTM1* and *GSTT1* nulls genotype are associated with an increased risk of lung cancer. In this study, we evaluated genetic polymorphisms in *CYP1A1* (**2B* and **4*), *GSTM1* and *GSTT1* and risk of lung cancer in Rebouças tunnel workers (Rio de Janeiro, Brazil). Deletions of *GSTM1* and *GSTT1* genes were detected in samples from control group. In an exposure group, single deletions of *GSTM1* or *GSTT1* genes were also detected. In our study, the same individual showed *CYP1A1*2B* polymorphism and deletions of *GSTM1* and GSTT1 gens. These results demonstrate that this individual exposed to occupational pollution at Rebouças tunnel, may have intensified metabolizing pollutants such as PAH, and have difficulties in detoxification of metabolites of these pollutants, increasing the risk of lung cancer development.

Keywords: lung cancer, Rebouças tunnel workers, polymorphisms, CYP1A1, GSTM1, GSTT1.

1 INTRODUCTION

Individual susceptibility to chemically induced cancer may be partly explained by genetic differences in the activation and detoxification of procarcinogens. Numerous polymorphisms of metabolism genes have been identified and their role in individual susceptibility to cancer has been shown in several studies [1], [2]. *CYP1A1* plays an important role in the metabolism of polycyclic aromatic hydrocarbons (PAHs), an important group of lung carcinogens [3], [4]. The CYP1A1 genes presents 8 polymorphisms [5], with three of them being in exon 7. *CYP1A1*2C* (A2455G) also termed *2B*, if it is in linkage disequilibrium with *2A* (T3801C), *CYP1A1*4* (C2453A) and *CYP1A1*5* (C2461A). Polymorphisms *CYP1A1*2B*, **4* and **5* lead to amino acid substitutions, which are in close proximity to the cysteine bound to the heme [5]–[7]. The first variant presents a Val instead of Ile at position 462 [5], [6], whereas the second presents an Asn instead of Thr at position 461 [5], [8], and the third presents Ser instead of Arg at position 464 [5], [9]. Catalytic activity studies have shown that the variants **2B* and **4* possess different catalytic activities when compared to the wild type protein [5], [10]. *CYP1A1* polymorphisms have been shown to increase microsomal catalytic activity for converting procarcinogens, including PAH and aromatic amines [4], [11]. Positive associations of *CYP1A1* genetic polymorphisms and lung cancer risk were pointed out in Japanese studies [4], [12].

Glutathione *S*-transferases (GSTs) belong to a superfamily of detoxication enzymes that provide critical defenses against toxicants [13]–[15]. Deletion polymorphism for the GST genes (especially the *GSTM1*) have been found to be associated with the development of lung cancer [15], [16], skin cancer [15], [17], bladder cancer [15], [18] and colon cancer [15], [19].

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Recent studies indicate that this polymorphism also plays a role in the susceptibility to adverse health effects from exposure to environmental pollutants [15], [20].

The human GST theta (*GSTT1*) gene, was recently isolated and sequenced by El-Zein et al. [15] and Pemble et al. [21]. Deletion polymorphism in this gene has been shown to modulate the toxicity of halogenated alkanes and epoxides in humans [15], [22], to influence the age of onset of colon cancer [15], [23] and to increase the risk for head and neck cancer [15], [24]. Recent evidence indicates that the *GSTT1* deletion polymorphism is also associated with increased risk to lung [15], [25].

Many studies have shown that variant *CYP1A1* alleles combined with *GSTM1* and *GSTT1* nulls genotype are associated with an increased risk of lung cancer [25]–[28].

Rebouças tunnel showed the highest concentrations of Polycyclic aromatic hydrocarbons (PAHs) and Nitro-Polycyclic aromatic hydrocarbons (nitro-PAHs), and the highest mutagenicity values for *Salmonella typhimurium* strain TA98 and its derivatives, YG1021 and YG1024, both sensitive to nitro-compounds [29], [30]. Our group conducted a biomonitoring study with Rebouças tunnel workers, in which significantly higher frequencies of MN were detected in binucleated lymphocytes and in cells of the buccal mucosa, besides higher concentrations of 2-naphthol and 1-hydroxyprene [31]. Damage to the genetic material and high concentrations of PAH metabolites were detected in biological samples taken from Rebouças tunnel workers and can be related to daily exposure to pollutants in the tunnel [31].

In this study, we evaluated genetic polymorphisms in *CYP1A1* (**2B* and **4*), *GSTM1* and *GSTT1* and risk of lung cancer in Rebouças tunnel workers (Rio de Janeiro, Brazil).

2 MATERIAL AND METHODS

2.1 Selection of exposure and control groups

The exposed group was formed by fifteen male workers tunnel Rebouças with age between 25 and 64 years. Peripheral blood samples were collected in tubes containing EDTA during the working day. After collection, the material was cooled and transported to our laboratory. The same procedures were carried out with volunteers in the control group, formed by eleven workers of the State University of Rio de Janeiro, male with age between 30 and 60 years. All procedures were submitted and approved by the National Research Ethics Committee – CONEP (CAAE Nº. 27402014.6.0000.5259).

2.2 Genetic polymorphism analysis of CYP1A1*2B and CYP1A1*4

The genetic polymorphism analysis for the *CYP1A1*2B and CYP1A1*4* were characterized by RFLP after PCR second (Cascorbi et al. [8]). DNA was extracted by KIT Pure Link® Genomic. All primers were obtained from New England Biolabs (Uniscience, Brazil); PCR reactions were performed with a Biorad Thermal cycler-MyCycler.

For determination of *CYP1A1*2B* DNA fragment was amplified using 1 unit Taq polymerase, 10 μmoI/liter of primers a 204-bp fragment with primers (reagent concentrations as above; PCR conditions were 35 cycles of 0.5 min at 94°C 0.5 min at 63°C, and 0.5 min at 72°C). The product was digested with BsrDI (New England Biolabs; 0.5 units). *CYP1A1*4* could be determined from the same 204-bp fragment but using BsaI (New England Biolabs; 2.5 units). Both cleavage sites were lost in the case of the mutations and were evaluated on Diamond and 1.5% agarose gel.

2.3 Genetic polymorphism analysis of *GSTM1* and *GSTT1* genes

The genetic polymorphism analysis for the *GSTMl* and *GSTTl* gens was determined simultaneously in a single assay using a multiplex PCR approach [15]. DNA was extracted by KIT Pure Link® Genomic. Briefly, isolated DNA (50 ng) was amplified in a 25 µl reaction mixture containing 0.5 mmol of each of the following *GSTM1* primers: in the presence of 0.2 mmol/L dNTPs and 1.5 mmol/L de $MgCl_2$.

The PCR conditions consisted of an initial melting temperature of 95°C (5 min) followed by 35 cycles of melting (94°C, 2 min), annealing (59°C, 1 min) and extension (72°C, 1 min). A final extension step (72°C) of 10 min terminates the process. The PCR products from amplification of *GSTT1* and *GSTM1* genes were then analyzed electrophoretically on Diamond and 1.5% agarose gel. The presence or absence of *GSTT1* and *GSTM1* gens was detected by the presence or absence of a band at 480 bp (corresponding to *GSTT1*) and a band at 215 bp (corresponding to *GSTM1*).

3 RESULTS

Polymorphism *CYP1A1* 2B* was detected in every individual exposure group. No *CYP1A1* polymorphism was detected in the control group (Fig. 1).

Deletions of *GSTM1* and *GSTT1* genes were detected in 100% of samples from control group. In an exposure group, single deletions of *GSTM1* or *GSTT1* genes were detected in 72.74% (n=11) to 86.37% (n=13), respectively (Fig. 2).

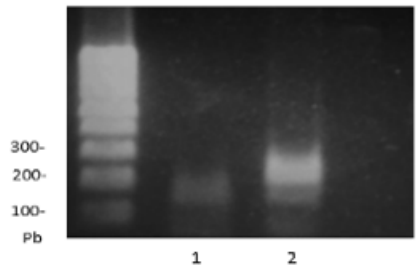

Figure 1: Agarose gel 1.5% show *CYP1A1*2B* polymorphism in an individual exposure group.

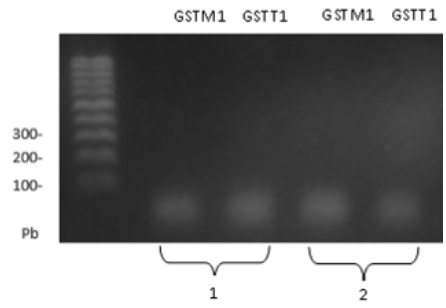

Figure 2: Agarose gel 1.5% show *GSTM1* and *GSTT1* polymorphisms in two individuals from an exposure group.

4 DISCUSSION

A number of studies have analyzed a possible association between *CYP1A1* polymorphisms with the risk of developing lung cancer [11]. In the present study, we analyzed samples from 15 workers Rebouças tunnel (total 50 workers), as genetic polymorphisms in *CYP1A1* (**2B* and **4*), *GSTM1* and *GSTT1*. Our results show a polymorphism *CYP1A1*2B* in an individual exposure group. The strong relationship between polymorphism *CYP1A1*2B* and lung cancer was first found in the Japanese population [8], [12]. *CYP1A1*2B* polymorphism was found in Asians at a high allelic frequency (0.22), followed by Latinos (0.16), and Caucasians (0.09), but not in Africans [5], [32]. In our population, the *CYP1A1*2B* polymorphism is present at the same allelic frequency as in Caucasians [5], [32]. Studies performed with Brazilian patients, *CYP1A1*2B* polymorphism also presented an increased risk of developing lung cancer [5], [33].

Our results showed deletions of *GSTM1* and *GSTT1* gens in 100% of samples from control group. In exposure group, deletions of *GSTM1* and *GSTT1* gens were detected in 72.74% to 86.37%, respectively. In a study by Rossini et al. [34] with volunteer residents of the city of Rio de Janeiro found that 42.1% of the individuals had the GSTM1 null genotype, whereas 25.4% had the GSTT1 null genotype. The prevalence of the deleted *GSTM1* genotype in North Americans was 51% and in Egyptians was 44% [15]. The prevalence of the deleted *GSTT1* genotype among North Americans was reported by Nelson et al. [35]. They reported that the prevalence of the null genotype was highest among African-Americans (21.8%) and Caucasians (20.4%), whereas the prevalence was lowest among Mexican-Americans (9.7%) [35].

As *CYP1A1* is a phase I enzyme that is involved in carcinogen activation and *GSTM1* and *GSTT1* is a predominant phase II enzyme for deactivation, they may be complementary in their modulation of cancer risk [28]. CYP1A1 takes part in the activation of PAHs into diol epoxide metabolites in the lung, and *GSTM1* and *GSTT1* plays an important role in the detoxification of diol epoxide metabolites [28]. Epidemiological studies on Asian population have shown an association between increased risk of lung cancer and the combination of the *GSTM1* null genotype and CYP1A1 variants [15]. In our study, the same individual showed *CYP1A1*2B* polymorphism and deletions of *GSTM1* and GSTT1 gens. These results demonstrate that this individual exposed to occupational pollution at Rebouças tunnel, may have intensified metabolizing pollutants such as PAH, and have difficulties in detoxification of metabolites of these pollutants, increasing the risk of lung cancer development.

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