GSTT1 gene deletion is associated with lung cancer in Mexican patients

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Abstract. Glutathione S-transferase (GST) is a dimeric detoxifying isoenzyme, involved in the deactivation of carcinogens, several tobacco-derived carcinogens, and xenobiotics. It catalyzes the reduction of glutathione to its thioester; thus, deficiency in GST activity due to homozygous deletion of the \textit{GSTT1} gene (null genotype) may play a role in the induction of lung cancer by smoking.

We studied the distribution of \textit{GSTT1} gene deletion in peripheral blood DNA samples from 178 healthy controls (41 nonsmokers, 63 passive smokers and 74 smokers) and 52 lung cancer patients. Comparisons between groups showed that there was an increased lung cancer risk for individuals with the \textit{GSTT1} null genotype. Cancer patients showed significant differences when compared with controls: nonsmokers, passive smokers, and smokers. Twenty-one percent of lung cancer patients carried the deletion versus 2% among nonsmokers not exposed to passive smoking, 6% among passive smokers, and 5% among smokers. Thus, there is a significant association between this genotype and the possibility to risk of developing lung cancer.

Keywords: Polymorphism, \textit{GSTT1}, lung cancer, Mexican population

1. Introduction

The glutathione S-transferases (GSTs; E.C.2.2.1.18) are families of multifunctional enzymes that mediate the conjugation of electrophilic compounds to glutathione, resulting in detoxification of some environmental carcinogens, pesticides and polycyclic aromatic hydrocarbons [3]. Five related polymorphic gene fam-
2. Materials and methods

Genomic DNA was extracted from peripheral blood samples according to standard protocols [8]. We recruited 178 healthy donors and 52 patients with lung cancer, all Mexicans living in Guadalajara City and its surroundings. All individuals signed a letter of informed consent. The cancer patients were diagnosed histologically in the Hospital of Oncology, Centro Medico Nacional de Occidente, Instituto Mexicano del Seguro Social (IMSS). Both groups were interviewed for personal data with a short questionnaire that included age, sex, detailed information on recent and past tobacco use, alcohol and any other type of drug consumption, occupational exposures and any history of the use of chemotherapy agents in the case of cancer patients. The subjects were classified as smokers (≥ 3 cigarettes per day), nonsmokers, passive smokers, and ex-smokers. The amount of tobacco smoke exposure was calculated as pack-years (packs–20 cigarettes–per day per year of smoking).

The presence or absence of the GSTT1 gene was determined by DNA amplification in a total polymerase chain reaction (PCR) volume of 25 μL containing 200 μM dNTPs, 25 ng of primers and 2.5 U Taq polymerase. We used the following primers: 5’- TTCCTTACTGGTCC TTCACATCTC-3’ and 5’- TCACCGGATCATGGCCAGCA-3’. These primers amplify the 3’ coding region of the human cDNA GSTT1 gene including a 480 bp segment. The PCR conditions consisted of an initial melting temperature of 94°C (4 min) followed by 35 cycles of melting (94°C, 2 min), annealing (59°C, 1 min), and extension (72°C, 1 min). The DNA samples were also amplified for a 312 bp segment of the CYP’A1 gene as an internal control. The samples were separated using 6% polyacrylamide gel electrophoresis (29:1) and were silver stained [12, 13]. Absence of the band indicates deletion of the gene, and presence of the band indicates a normal allele. To confirm the absence of the GSTT1 gene in the samples, we amplified exons 6–7 (5’- CCATG- GTTTGCAGGAAACAAG-3’ and 5’- GACGAAATCTACAAAAGT-3’), 2–3 (5’- AAGAAGTACACGATGGGGAC-3’ and 5’- CTGGCTTCTGTCATAATCAGG-3’), and 5–6 (5’- AGACGAAGAGGAGAAGATTCC-3’ and 5’- TCCAGTACCTTTGGCTTCAGT-3’) of GSTT1 and the results were negative for these amplified fragments [14].

The values were analyzed using Chi-squared tests. P < 0.05 was used as the criterion for statistical significance. Yates corrected tests were used to test the significance of proportions, with 95% confidence intervals (C.I.), to estimate the odds ratio common to different levels and to test whether this was equal to one.

3. Results and discussion

Table 1 presents the characteristics of the lung cancer patients and controls. The frequency of GSTT1
deletion was 5.0% among the population controls, and 21.0% among the lung cancer patients. The lung cancer patients showed a significantly higher frequency of $GSTT1$ deletion with an odds ratio of 5.04 (95% confidence intervals, 1.8–14.3), and Yates corrected test $P < 0.01$. The genotype distributions from cases and controls were all consistent with Hardy-Weinberg equilibrium that was analyzed by the likelihood ratio.

Table 2 shows the overall distribution of cases and controls according to genotype for $GSTT1$ deletion. The lung cancer patients with the deletion included one passive smoker with a histological diagnosis of adenocarcinoma, and one undefined. Of the patients who had smoked 15 pack-years or less, only one had undefined histology. Eight of the cancer patients reported smoking 31 pack-years or more (five had epidermoids, two had adenocarcinomas and one was undefined).

Cancer patients with presence of the $GSTT1$ gene included five passive smokers (three undefined, one with an adenocarcinoma and one with an epidermoid cancer), and 36 who smoked 31 pack-years or more (nine undefined, nine with adenocarcinomas, 12 with epidermoids and six with small cell cancers).

We found that 21% (95% confidence interval of 1.8 to 14.3) of lung cancer patients have a deletion of the $GSTT1$ gene and 5% of healthy controls have deletion of $GSTT1$ gene. For cancer screening, it is important that the fraction of controls with a marker be very small to avoid large numbers of unnecessary interventions [15]. Thus the $GSTT1$ deletion phenotype has promise, if combined with another marker to improve performance, in the early detection and screening of individuals at a high risk for lung cancer in the Mexican population.

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