Lack of association of \textit{CYP1A1-MspI} SNP and \textit{GSTM1} null genotypes with cancer in a Brazilian family with unusually high cancer incidence

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Received March 2, 2011
Accepted May 14, 2012
Published June 15, 2012
DOI http://dx.doi.org/10.4238/2012.June.15.10

\textbf{ABSTRACT.} Research has shown that genetic polymorphisms in biotransformation enzymes, such as CYP1A1 and GSTM1, are related to a greater or lesser susceptibility to various cancers. We made an analysis of \textit{CYP1A1m1} SNP and \textit{GSTM1} null genotypes in a family group (71 members) related by consanguinity who had an unusually high incidence of cancer and a high frequency of smokers. There were no significant differences in genotype frequencies in this family when compared to data for Brazilian populations. Possibly, the high incidence of cancer in this sample is associated with smoking and/or other factors not detected in this survey.

\textbf{Key words:} \textit{CYP1A1m1} SNP; \textit{GSTM1} null; Brazilian families; Cancer
INTRODUCTION

The genetic variability of the capacity for the biotransformation of xenobiotics has been associated with greater or lesser susceptibility to neoplastic development. The main gene families that metabolize endogenous and exogenous xenobiotics activating them in phase I are cytochrome P450, e.g., $CYP1A1$, and the glutathione S-transferase (GST) family, which acts in phase II, e.g., $GSTM1$.

The $CYP1A1$ gene is located on chromosome 15q24.1 (Hildebrand et al., 1985; Kawajiri et al., 1986) and codes for an enzyme that links hydrophilic groups to the polycyclic aromatic hydrocarbons (PAHs) and results in reactive molecules for the subsequent conjugation reaction (Rebbeck, 1997). Exposure to benzo[a]pyrene, a powerful precarcinogen present in cigarette smoke and highly capable of forming covalent links with DNA (Duarte and Paschoal, 2005), and other PAHs induce the expression of the $CYP1A1$ gene (Hirvonen, 1995). One of the single nucleotide polymorphisms (SNPs) of the $CYP1A1$ gene most widely studied is the $MspI$ restriction site ($CYP1A1m1$) in the 3’-non-coding region, a T→C transition that promotes an increase in the expression of the gene (Nakata et al., 2004). The high activity of this gene can be deleterious and contribute to the susceptibility to some diseases, including endometriosis (Nakata et al., 2004), cancers associated with tobacco (Cha et al., 2007), cardiovascular diseases, type 2 diabetes mellitus (Wang et al., 2002), and leukemia (Vineis and Perera, 2007).

The $GSTM1$ gene is located on chromosome 1p13.3 (Rebbeck, 1997). The majority of GSTs exist as soluble enzymes in the cytoplasm. The regulation of $GSTM1$ is subject to a set of complex endogenous and exogenous parameters including gender, organ, and a great number of inductive xenobiotic agents, such as PAHs, phenolic antioxidants, reactive oxygen species, isothiocyanates, barbiturates, and glucocorticoid synthetics (Eaton and Bammler, 1999). Glutathione S-transferase catalyzes nucleophilic attacks by glutathione (GSH) on electrophilic substrates, reducing their reactivity with intracellular biomolecules (Eaton and Bammler, 1999). GSTM1 catalyzes phase II of the detoxification of PAHs, such as the precarcinogen benzo[a]pyrene (Hatagima, 2002; Oga et al., 2003). Individuals who show homozygosis due to the deletion of the $GSTM1$ gene have a diminished capacity to eliminate xenobiotics metabolically and, as a consequence, an increased risk of developing neoplasms. The null genotype (-/-) has been associated with the development of diverse types of cancers, such as lung (Hirvonen et al., 1993), colorectal (Zhong et al., 1993; Deakin et al., 1996), bladder (Lin et al., 1994; Santella et al., 1995), colon, and stomach adenocarcinoma, carcinoma of the oral cavity (Sato et al., 1999) and prostate cancer, and sensitivity to various chemical compounds (Schnakenberg et al., 2007).

The production of cytosolic, unstable and reactive molecules is lower in individuals with the homozygous wild genotype (wt/wt) when compared with genotypes wt/m1 and m1/m1, which have increased expression or overexpression of the $CYP1A1$ gene (Landi et al., 1994). Thus, GSTM1 null individuals (-/-), i.e., without this phase II biotransformation enzyme, have greater difficulties in disposing of some reactive molecules produced in phase I. Some reports associate this genotype with a greater predisposition to develop certain neoplasms. People who have null genotypes for GSTM1 (-/-) and also display an overproduction of $CYP1A1$ (m1/m1 or wt/m1), an enzyme of phase I, will have an increase in candidate molecules for DNA adducts, increasing the predisposition for the development of certain types of cancer (Autron, 2000; Arvanitis et al., 2003).
In the present study, a diagnosis of CYP1A1m1 and GSTM1*0 genotypes from samples of blood-related persons with well-known cancer incidence was carried out.

MATERIAL AND METHODS

Seventy-one individuals from a Brazilian family with a history of consanguinity and recurrent occurrence of cancer were analyzed. The research included living individuals who agreed to donate biological material and answer a short questionnaire. We collected around 3 mL peripheral blood in heparinized tubes. The samples were identified and stored at 4°C.

DNA extraction was carried out by the method of Lahiri and Nurnberger (1991), according to the following steps: a) 300 µL blood was added to a 1.5-mL conical tube containing 1 mL lysis buffer 1 (10 mM Tris-HCl, pH 7.6, 10 mM KCl, 10 mM MgCl₂, 2 mM EDTA, 2.5% Triton X-100) and mixed by inversion for 5 min; b) the tube was centrifuged at 5000 rpm for 5 min; c) the supernatant was discarded and the pellet resuspended in 1 mL 1X wash buffer (TKM1 = 10 mM Tris-HCl, pH 7.6, 10 mM KCl, 10 mM MgCl₂, 2 mM EDTA); d) the tube was centrifuged at 5000 rpm for 5 min, and steps a, b and c were repeated; e) the supernatant was discarded and 200 µL lysis buffer 2 (10 mM Tris-HCl, pH 7.6, 10 mM KCl, 10 mM MgCl₂, 2 mM EDTA, 400 mM NaCl) was added plus 20 µL 10% SDS; f) the cells were resuspended and incubated at 65°C for 10 min; g) 50 µL 5 M NaCl was added and the tube mixed by inversion; h) the sample was centrifuged at 10,000 rpm for 10 min, and the supernatant transferred to a clean tube, discarding the precipitate; i) 1 mL (2.5 volumes) cold absolute ethanol was added and the tube mixed by inversion; j) the tube was centrifuged at 10,000 rpm for 10 min and the supernatant discarded, k) DNA was washed in 70% ethanol and centrifuged at 10,000 rpm for 3 min; l) the supernatant was discarded, leaving the DNA precipitate dry; m) after drying, the DNA precipitate was dissolved in 30 µL ultrapure water and stored at -20°C. The polymerase chain reaction (PCR) method for detection of polymorphic CYP1A1 genotypes was used. The product was digested by MspI to investigate the m1 polymorphic allele by RFLP (restriction fragment length polymorphism). The primers for CYP1A1 were the same as used by Sarmanová et al. (2001): CYP1A1-F (5'-CAGTGAAGAGGTGTAGCCGCT-3'), CYP1A1-R (5'-TAGGAGTCTTGTCTCATGCCT-3'). DNA (4 ng/µL), 0.08 µM of each primer, 0.2 mM of each dNTP, 1X buffer, 2 mM MgCl₂ and 0.025 U/µL Taq DNA polymerase were mixed. The reaction was performed with the following temperature scheme: a) 4 min at 94°C; b) 1 min at 94°C; c) 1 min at 57°C; d) 1 min at 72°C; e) return to step b 35 times; f) 10 min at 72°C, and g) stop the reaction. The PCR products were digested by MspI and the resulting fragments separated on a 10% polyacrylamide gel and stained with silver nitrate. The wild-type allele (wt) was not digested by the enzyme and showed a fragment of 340 bp, since the polymorphic allele (m1) was digested into fragments of 200 and 140 bp. The heterozygous displayed the three fragments (340, 200 and 140 bp) (González et al., 2004).

The primers used in PCR for the detection of GSTM1 genotypes were designed by Zhong et al. (1993), GSTM1-F (5'-CGCCATCTTGTGCTACATTGGC-3'), GSTM1-R (5'-TTCTGGATGAGGCACCATGACAC-3'), and amplified a 230-bp product that is specific for the GSTM1 gene. The GSTM1 null genotype (-/-) did not show the product. We used the primers GSTM4-F (5'-CGCCATCTTGTGCTACATTGGC-3') and GSTM4-R (5'-ATCTTTCTCTCTCTCTTCTC-3'), which generate a product of 157 bp, which was used as the positive control reaction. The reagents used in the reaction were 4 ng/µL DNA, 0.08
µM of each primer, 0.2 mM of each dNTP, 1X buffer, 2 mM MgCl₂, and 0.025 U/µL Taq DNA polymerase. The reaction was performed with the following temperature scheme: a) 4 min at 94°C; b) 1 min at 94°C; c) 1 min at 52°C; d) 1 min at 72°C; e) return to step b 35 times; f) 10 min at 72°C, and g) stop the reaction. The products were visualized on a 1.8% agarose gel stained with ethidium bromide. This study was approved by the Ethics in Research of Hospital Julio Miller, Universidade Federal de Mato Grosso (#360/CEP/HUJM/07).

RESULTS AND DISCUSSION

The determination of the \textit{CYP1A1-MspI} SNP and \textit{GSTM1}-positive and null genotypes is shown in the pedigree chart in Figure 1. One homozygous \textit{m1/m1} (1.41%), 28.17% heterozygous (wt/m1) and 70.42% wild-type homozygous \textit{wt/wt} were found (Table 1). The \textit{m1} allele frequency was 15.49%. These results are very close to those reported by Gaspar et al. (2004), who analyzed a population of European descent from the city of Porto Alegre (Brazil), in which the frequency of the \textit{m1} allele was 17%.

![Pedigree chart showing the family studied. Above the diagonal in the circles and squares are the genotypes for \textit{CYP1A1}, with the colors white (\textit{wt/wt}), gray (\textit{wt/m1}) or black (\textit{m1/m1}). Below the diagonal in the circles and squares are genotypes for \textit{GSTM1}, with the colors white (+/+ or +/-) or black (-/-). The gray circles and squares with no diagonal represent those who were not volunteers in this study. The letter C indicates individuals with cancer.](image-url)

\begin{table}[h]
\centering
\begin{tabular}{lccc}
\hline
\textit{GSTM1} & \textit{wt/wt} & \textit{wt/m1} & \textit{m1/m1} \\
\hline
+/+ & 40.85 & 19.72 & 1.41 & 61.97 \\
+/- & 29.58 & 8.45 & 0.0 & 38.03 \\
-/- & 19.55 & 8.45 & 1.41 & 30.00 \\
\hline
Total & 70.42 & 28.17 & 1.41 & 100.0 \\
\end{tabular}
\caption{Genotype frequencies of the \textit{CYP1A1-MspI} SNP and \textit{GSTM1} positives and nulls from a sample of 71 individuals from a Brazilian family.}
\end{table}
Frequencies of 38.03% for the null genotype (-/-) and 61.97% for the positive genotype [heterozygous (+/-) and positive homozygous (+/+)] for the GSTM1 gene were observed (Table 1). These values are also close to that expected in samples of Brazilian populations (Miller et al., 1997; Arruda et al., 1998; Hatagima et al., 2000; Rossini et al., 2002; Maciel et al., 2009). Members of this homozygous sample CYP1A1-MspI (m1/m1) who were also homozygous GSTM1 null genotype (-/-) were not found.

This research shows a genetic structure lineage initiated by paternal individuals who were born in the nineteenth century. Individual I-1 was born in 1863 and married individual I-2, who was born in 1870. Individual I-3 was an Italian born in 1870, who came to Brazil as a teenager and who married the young I-4, who was also born in 1870. Individual I-5 was born in 1870 and his first wife (I-6) was also his cousin and together they had three children. Individuals I-2 and I-5 were also cousins. Individual I-5 had three other sons with his second wife (I-4, who was a cousin of II-14). Individuals III-4 and III-5 were also cousins.

Information on cancer occurrence was obtained through questionnaires distributed to family members, who agreed to take part in research. Therefore, it was possible to cross-check information from different interviewees and to confirm cases of cancer among the relatives (Figures 1 and 2). Twenty-one individuals (22.68% included in the pedigree in Figure 1) showed some type of cancer. Skin cancer was the most frequent with 9 cases, followed by the occurrence of 4 cancers of the lung, 3 of the neck, 2 of the stomach, 2 of the intestine, 1 of the mouth, 1 of the head, and 1 of the liver, as shown in Figure 2.

![Cancers in the family](image)

**Figure 2.** Data from 21 cancer cases reported in the pedigree in Figure 1. Skin: II-(1), III-(6, 7, 22, 23, 24), IV-(15, 18, 30); intestine: II-(1, 5); liver: II-(9); head: II-(10); stomach: II-(12), III-(30); throat: III-(3, 12, 27); lung: III-(9, 11, 13, 25); mouth: IV-(15).

The reports of cancer in the family showed differences in the frequency of this disease, when considering the sample for six generations (Figure 1). The occurrence of cancer cases in generation I was not detected. Of the 13 individuals considered to be within generation II, only one was alive and was a research volunteer. It was not possible to know about the cancer occurrence in some individuals of this second generation, even though 5 cases were detected. Generation III, which is the oldest living generation, had the largest number of cases (13), perhaps due to advanced age of the sample persons, whose average age was 70 years. The
smaller number of cancer in generation IV (3 subjects) and none in generation V is possibly due to the individuals’ lower average age, i.e., 47 and 24 years, respectively.

The volunteers responded to a questionnaire, where they would report if they smoked, how long has the respondent been smoking or has smoked, and how long he/she has gone without smoking. Cigarette smoking was recorded only among the volunteers, yet nearly 40% of the volunteers said they smoke (Figure 3). A familial relationship with smoking was observed. The analysis of the questionnaire also detected that many of those who referred to themselves as non-smokers, reported that they are passive smokers because they are married to smokers.

The consensus is that most cancers are theoretically preventable, as several epidemiological studies suggest that a large number of cancers develop due to genetic conditions interacting with environmental factors, such as sporadic or frequent exposure to xenobiotics, radiation, and viruses. On the other hand, smoking and diet are also important targets of campaigns to prevent cancer (Sugimura, 2000).

Consanguinity, occurrence of cancer, and high number of smokers in this family prompted us to diagnose the genotypes CYP1A1 MspI SNP and GSTM1 null among the living members who opted to participate as research volunteers. The results did not reveal significant differences in genotype frequencies within this family when compared to the results obtained by the analysis of samples of Brazilian populations. Perhaps the high incidence of cancer in this sample is associated with smoking and/or other factors not detected by this survey.

ACKNOWLEDGMENTS

Research supported by Fundação de Amparo à Pesquisa do Estado de Mato Grosso (FAPEMAT; #738368/2008).
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