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Short Communication

Glutathione S-transferase Mu (GSTM1) null genotype in relation to gender, age and smoking status in a healthy Brazilian population

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Abstract

The glutathione S-transferase mu (GSTM1) gene which acts during phase II of xenobiotic metabolism is polymorphic in the population, being absent in about 30-50% of individuals depending on the ethnic group from which they come. Epidemiological studies suggest that individuals who are homozygous null at the GSTM1 locus may have an increased risk of developing various types of neoplastic disease. We used the polymerase chain reaction (PCR) to estimate the frequency of GSTM1 in 176 healthy individuals from the north of Paraná (Brazilian state), the null genotype being detected in 48.86% of these individuals. The Student's t-test was used to evaluate the frequency of the glutathione S-transferase null genotype in relation to age, gender and smoking habit and no significant differences were found. In our sample there were 142 individuals of Caucasian origin, of which 47.88% had the null genotype. When applied to the Caucasian group only (n = 142) the Student's t-test again showed no significant differences between the frequency of the GSTM1 null genotype and age, gender and smoking habit.

Key words: glutathione S-transferase Mu (GSTM1), control individuals, Brazilian population, age, smoking habit.

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Introduction

The differential distribution of variant polymorphic genes in different human populations around the world may influence the environmental diseases which they acquire (Au *et al*. 1999). The frequency of the GSTM1 null genotype in humans ranges from 30-50% depending on the ethnic origin of the individual (London *et al.* 1995). Epidemiological studies suggest that individuals who are homozygous null at the GSTM1 locus (i.e. lack both copies of the GSTM1 variant of the GST gene) may have an increased risk of developing various types of neoplastic disease, including cancer of the bladder, colon, skin, lung and stomach (Ford *et al*. 2000; Harada *et al*. 1987; Hayes and Pulford 1995; Johns and Houlston 2000; Raunio *et al*. 1995; Zhong *et al*. 1993a). Therefore, the knowledge of inheritance of these susceptible genes in different ethnic groups is very important for effective disease prevention, especially against cancer (Au *et al*. 1999).

Many of the first reports on genetic risk modification came from Japan, and, after several studies, genotypes associated with cancer risk have also been obtained for Caucasian and other population (Raunio *et al*. 1995). The frequency of the GSTM1 null genotype in healthy populations ranges from 23-41% in African-Americans and Blacks, 33-69% in Asiatics, 39-62% in Europeans and 51-54% in Australians. The highest frequencies (64-100%) have been reported in studies involving small numbers of subjects from parts of the South Pacific (Cotton *et al*. 1999). The variations in GSTM1 expression among control populations presents great difficulties for researchers in the selection of suitable control groups to be used for determining the association between GST genotype and disease susceptibility (Hayes and Pulford 1995).

The ethnic origin of the Brazilian population is highly heterogeneous, the population being composed of Indigenous peoples and immigrants from Europe, Africa and Asia (Arruda *et al*. 1998). Studies on healthy Brazilians have shown a frequency of the GSTM1 null genotype of 33% (D'Arce and Cólus, 2000) and 41.2% (Cabral *et al*., 1999).

Currently there has been great interest in studies of genes which code for enzymes able to metabolize xenobiotics, although studies involving Brazilian populations are rare. The aim of our study was to estimate the frequency of the GSTM1 gene in a group of healthy Brazilians from the state of Paraná and characterize the sample according to the presence or absence of this gene regarding gender, age and smoking habits.

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Materials and Methods

Population studied

We used blood samples from 176 people born in the north of the southern Brazilian state of Paraná, the sample consisting of individuals whose ancestors were Caucasians (n = 142), Africans (n = 13), Asians (n = 10) and Brazilian Indians (n = 5). It was not possible to determine the ethnic group which belong some individuals (n = 6), due to doubtful informations in the questionnaire. Those individuals, however, have been analyzed in the total sample. After the subjects' had given their consent, personal information regarding life style, age, marital status, ethnic group, occupational history, smoking habits, family history of cancer and others factors was obtained according to the procedures of Carrano and Natarajan (1988).

Genotype analyses

DNA was isolated from the peripheral white blood cells of all the individuals sampled using the technique described by Miller *et al*. (1988) and the GSTM1 genotype determined by a modification of the polymerase chain reaction (PCR) procedure described by Topinka *et al*. (1997). The oligonucleotide primers (P1, P2, P3) used for the PCR were 5'-CGC CAT CTT GTG CTA CAT TGC CCG-3' (P1), 5'-ATC TTC TCC TCT TCT GTC TC-3' (P2) and 5'-TTC TGG ATT GTA GCA GAT CA-3' (P3). The three primers were used together in the reaction and formed two couples as follows: pair P1 and P2 (which anneal with homologous sequences of genes GSTM1 and GSTM-4) yielded one 157 bp fragment used as internal control for the reaction and pair P1 and P3 (specific for the polymorphic gene GSTM1) produced a fragment of 230 bp (Figure 1). The PCR was carried out in a volume of 25 µL containing 10 mM pH 8.3 Tris-HCL, 50 mM KCL, 1.0 mM $MgCL_2$, 5% DMSO, 2.5 mM of each dNTP, 50-200 ng of genomic DNA, 1.5 units of Taq DNA polymerase (Life Technologies, Bethesda, MD, USA), 200 mM of primer 1 and 100 mM each of primers 2 and 3. Incubation was for 35 cycles of 94 °C at 1 min, 52 °C at 1 min and 72 °C at 1 min. The reaction was amplified using a thermal cycler PTC-100 (M.R. Research) and the PCR products separated by electrophoresis in 1.8% agarose gel and visualized by staining with ethidium bromide (10 mg/mL).

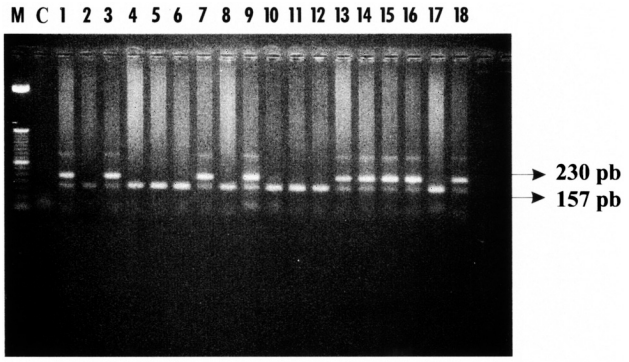

Figure 1 - GSTM1 gene PCR products resolved by agarose gel electrophoresis. M is a 50 bp DNA Ladder and C is the Control. A 157 pb DNA fragment can be seen in all the PCR reactions and a 230 bp DNA fragment is only present in samples containing the GSTM1 gene.

Statistical analyses

The Student's t-test was used to test the relationship between the GSTM1 null genotype and the smoking habits, age and gender of individuals from the total sample (n = 176) and the Caucasian subsample (n = 142). Statistical analysis was carried out using the Statgraphics (1989 version) software package (Statistical Graphics Corp.).

Results

The frequency of the GSTM1 null genotype for the total sample (n = 176) was 48.86% and 47.88% for the Caucasian subsample (n = 142). No statistical differences were detected in regard to age, gender and smoking habits in either the total sample (Table I) or the Caucasian subsample (Table II).

Discussion

Many genetic polymorphisms in metabolic enzymes are important risk factors in cancer, as has been shown in the large number of case-control studies which have been undertaken and the relative risk estimates have shown large variations between such populations studies (Knudsen *et al*. 2001). The GSTM1 gene acts during phase II of xenobiotics metabolism and its correspondent enzyme plays an important role in detoxifying chemical compounds, including polyaromatic hydrocarbons, such as those found in cigarettes, foods, pollutants, agrochemicals, chemotherapeutic drugs and oxidative stress products (Conde *et al*. 1999; Hirvonen 1995; London *et al*. 1995; Zhong *et al*. 1993b).

Table I - Number of individuals and frequency (%) of the GSTM1 null genotype in the total sample according to age, gender and smoking habits.

	Age			Gender		Smoking habits		Total
	17-30	31-50	>50	Male	Female	Non-smokers	Smokers	
Number of individuals	87	72	17	85	91	151	25	176
GSTM1 null frequency (%)	43.67	51.38	58.82	47.05	50.54	49	48	48.86

Table II - Number of individuals and frequency (%) of the GSTM1 null genotype in the Caucasian subsample according to age, gender and smoking habits.

	Age			Gender		Smoking habits		Total
	17-30	31-50	>50	Male	Female	Non-smokers	Smokers	
Number of individuals	79	50	13	61	81	124	18	142
GSTM1 null frequency (%)	41.77	54	69.23	49.18	48.14	50	38.88	47.88

Brazil is a large and highly heterogeneous country in regard to population, and differences in genic frequencies for control populations are easily found in different regions. The frequency of the GSTM1 null genotype (48.86%) which we found in the 176 healthy individuals of our sample drawn from the South of the country is similar to the frequency obtained by Buim (1999) and Cabral *et al*. (1999) in studies with healthy individuals from the Southern and Northern regions of Brazil as well as that obtained with other control populations (Zhong *et al*. 1993b). However, a lower GSTM1 null genotype frequency (36.9%) was reported by Arruda *et al*. (1998) in a Brazilian control population from the North, Northeast and Southeast.

We also analyzed the frequency of the GSTM1 null genotype according to gender, age and smoking habits. The GSTM1 gene was absent in 47.05% of the men and 50.54% of the women, but this difference was not statistically significant, this being similar to the results obtained by Alexandrie *et al*. (1994) and Kihara *et al*. (1994). The frequencies we obtained for the three age groups were 43.67% for the 17-30 group, 51.38% for the 31-50 group and 58.82% for the >50 group. Rossit *et al*. (1999) in a GSTM1 gene polymorphism study with Brazilians from 15-60 year-old, obtained a frequency of 47.7% for the null genotype. In our study, the null genotype seems to increase with age, although this was not statistically significant. Additional studies are necessary to confirm this tendency because we had no individuals younger than 17 years old in our study.

Genetic polymorphisms of metabolic enzymes have been demonstrated as an important risk factor in the development of cancer when combined with exposures of tobacco smoke (Knudsen *et al*. 2001). According to Kihara *et al*. (1994), the development of lung cancer depends on the smoking habit and the null GSTM1 genotype is one of the genetic factors involved in the individual response to smoke exposure and tumor development. Thus, the elaboration of studies to determine the frequency of this gene in control populations is of great importance to cancer prevention.

Different ethnic groups show differences regarding the presence and the absence of the GSTM1 gene which may influence the efficiency and interpretation of epidemiological studies, and because of this there is a great need to study the frequency of this gene in different ethnic groups (Lin *et al*. 1994).

The ethnic distribution of the GSTM1 null genotype has been studied mainly in Caucasian populations and, for this ethnic group, the frequency of the null genotype (47.88%) obtained in our study is similar to that described in the literature for healthy white populations of different nationalities (Gawronska-Szklarz *et al*. 1999; Lin *et al*. 1994; Stücker *et al*. 1999). Nevertheless, Arruda *et al*. (1998) and Gattás and Soares-Vieira (2000) have reported the absence of the GSTM1 gene in 55% and 60.2% of Caucasians in populations from Southeast Brazil.

In our study, no statistical differences were found regarding the absence of the GSTM1 gene according to gender, age and smoking habit among the Caucasians, corroborating the results described by London *et al*. (1995) who found no statistical differences for the null genotype in respect of any of these parameters for a Caucasian population from the USA.

The present study regarding the determination of the GSTM1 gene frequency in a Brazilian control population is an important contribution for future bio-monitoring studies which aim to assess risk from exposure to xenobiotics , especially because there is little data in the literature regarding Latin America populations in general and Brazilian populations in particular. Data obtained from different regions of Brazil may prove useful in explaining the different immigration patterns that occurred in the various regions of Brazil and for national epidemiological studies.

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References

Alexandrie A, Sunderberg MI, Seidegard J, Tornling G and Rannug A (1994) Genetic susceptibility to lung cancer with special emphasis on CYP1A1 and GSTM1: a study on host factors in relation to age at onset, gender and histological cancer types. Carcinogenesis 15:1785-1790.

Arruda CVR, Grinolli ME, Gonçalves MS, Soares MC, Menezes R, Saad STO and Costa, FF (1998) Prevalence of

homozygosity for the deleted alleles of glutathione S-transferase mu (GSTM1) and theta (GSTT1) among distinct ethnic groups from Brazil: relevance to environmental carcinogenesis? Clin Genet 54:210-214.

Au WW, Torres CHS, Salazar NC and Salama AS (1999) Inheritance of polymorphic metabolizing genes and environmental disease and quality of life. Mutat Res 428:131-140.

Buim ME (1999) Fatores genéticos na resposta à exposição ocupacional de agricultores Paranaenses a agroquímicos. Master Dissertation, Universidade Estadual de Londrina, Londrina.

Cabral IR, Rossit AR and Hamel AR (1999) Polimorfismos em genes de biometabolismo e prevalência do alelo CYP2E1*c2 em deficientes de G6PD assintomáticos: análises em uma população paraense. Genet Molec Biol 22 (suppl): 236-237. IV Congresso da Sociedade Brasileira de Mutagênese, Carcinogênese e Teratogênese Ambiental, Águas de Lindóia, Brazil.

Carrano AV and Natarajan AT (1988) Considerations for population monitoring using cytogenetic techniques. Mutat Res 204:381.

Conde AR, Martins G, Saraiva C, Rueff J and Monteiro C (1999) Association of P53 and glutathione S-transferase null genotype in gastric cancer in Portuguese population. Clin Mol Pathol 52:131-134.

Cotton SC, Sharp LJ and Brockton N (1999) Glutathione S transferase polymorphisms and colorectal cancer. Am J Epidemiol 1-18.

D'Arce LPG and Cólus IMS (2000) Cytogenetic and molecular biomonitoring agricultural workers exposed to pesticides in Brazil. Teratog Carcinogen and Mutagen 20:161-170.

Ford JG, Li Y, O'Sullivan MM, Demopoulos R, Garte S, Taioli E and Rauf PWB (2000) Glutathione S-transferase M1 polymorphism and lung cancer risk in African-Americans. Carcinogenesis 21:1971-1975.

Gattás GJF and Soares-Vieira JA (2000) Cytochrome P450-2E1 and Glutathione S-transferase mu polymorphisms among Caucasians and mulattoes from Brazil. Occup Med 50:508-511.

Gawronska-Szklarz B, Wójcicki M, Kuprianowicz A, Kedzierska K, Kedzierski M, Górnik W and Pawlik A (1999) CYP2D6 and GSTM1 genotypes in a Polish population. Eur J Clin Pharmacol 55:389-392.

Harada S, Abei M, Tanaka N, Agarwal DP and Goedde HW (1987) Liver glutathione S-transferase polymorphism in Japanese and its pharmacogenetic importance. Hum Genet 75:322-325.

Hayes JD and Pulford DJ (1995) The Glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 30:445-600.

Hirvonen A (1995) Genetic factors in individual responses to environmental exposures. J Occup Environ Med 37:37-41.

Johns LE and Houlston RS (2000) Glutathione S-transferase μL (GSTM1) status and bladder cancer risk: a meta-analysis. Mutagenesis 15:399-404.

Kihara M, Kihara M and Noda K (1994) Lung cancer risk of GSTM1 null genotype in dependent on the extent of tabaco smoke exposure. Carcinogenesis 15:415-418.

Knudsen LE, Loft SH and Autrup H (2001) Risk assessment: the importance of genetic polymorphisms in man. Mutat Res 482: 83-88.

Lin HJ, Han CY, Bernstein DA, Hsiao W, Lin BK and Hardy S (1994) Ethinic distribution of the glutathione transferase Mu 1-1 (GSTM1) null genotype in 1473 individuals and application to bladder cancer susceptibility. Carcinogenesis 15:1077-1081.

London SJ, Daly AK, Cooper J, Navidi WC, Carpenter CL and Idle JR (1995) Polymorphysm of glutathione S-transferase M1 and lung cancer risk among African-American and Caucasians in Los Angeles County, California. J Natl Cancer Inst 87:1246-1253.

Miller AS, Dykes DD and Polesky HF (1988) A simple salting out procedure for extraction DNA from human nucleated cells. Nucleic Acid Res 16:1215.

Raunio H, Pursiainen KH, Anttila S, Hietanen E, Hirvonen A and Pelkonen O (1995) Diagnosis of polymorphisms in carcinogen-activating and inactivating enzymes and cancer susceptibility- a review. Gene 159:113-121.

Rossit ARB, Cabral IR, Hamel AR and Conforti-Froes NDT (1999) Estariam as freqüências de genes do biometabolismo sujeitas à pressão seletiva durante o desenvolvimento humano? Genet Molec Biol 22 (suppl): 218. IV Congresso da Sociedade Brasileira de Mutagênese, Carcinogênese e Teratogênese Ambiental, Águas de Lindóia, Brazil.

Stücker I, Waziers I, Cenée S, Bignon J, Depierre A, Milleron B, Beaune P and Hémon D (1999) GSTM1, smoking and lung cancer: a case control study. Int J Epidemiol 28:829-835.

Topinka J, Binkova B, Mrackkvá G, Stávková Z, Peterka V, Benes I, Dejmek J, Lenícek J, Pilcík J and Srán RJ (1997) Influence of GSTM1 and NAT2 genotypes on placental DNA adducts in a environmentally exposed population. Environ Mol Mutagen 30:184-195.

Zhong S, Wyllie AH, Barnes D, Wolf CR and Spurr NK (1993a) Relationship between the GSTM1 genetic polymorphism and susceptibility to bladder, breast and colon cancer. Carcinogenesis 14:1821-1824.

Zhong S, Spurr NK, Hayes JD and Wolf CR (1993b) Deduced amino acid sequence, gene structure and chromosomal location of a novel human class Mu glutatione S-transferase, GSTM4. Biochem J 291:41-50.