

Full Length Research Paper

Glutathione-S-transferase (M1 and T1) polymorphisms in Nigerian populations

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The Mu (μ) and Theta (Θ) classes of glutathione transferase gene, GSTM1 and GSTT1 are polymorphic in humans. GSTM1 and GSTT1 enzyme deficiencies have been suggested to predispose people to lung, bladder and colorectal cancer. This study was carried out to investigate the distribution of Glutathione transferases (GSTs) genotype frequency in the three major Nigerian ethnic groups with a view to understanding the implication of the GST polymorphisms on disease susceptibility. Three hundred unrelated volunteers from the three major Nigerian ethnic groups of Hausa, Ibo and Yoruba who consented to the study were genotyped for GSTM1*1, GSTM1*0, GSTT1*1 and GSTT1*0 by polymerase chain reaction (PCR) technique. The homozygous null GSTM1*0 genotype frequency was found to be 37% in Hausa, 23% in Ibo and 31% in Yoruba with significant difference ($p < 0.05$) especially, in the Ibo ethnic group while GSTT1*0 genotype was found at a comparably high frequencies of 42, 36, and 35% in the Hausa, Ibo and Yoruba populations of Nigeria, respectively. The genotype frequencies of the null GSTM1 and GSTT1 were found to be relatively high in the Nigerian population with a possible implication on increase susceptibility to some cancers.

Key words: Genetic polymorphisms; genotyping, Nigerian populations, Glutathione-S-transferases.

INTRODUCTION

Glutathione transferases (GSTs) are a multigene family of enzymes that protect cells against electrophiles and products of oxidative stress. They catalyze the nucleophilic attack of the tripeptide thiol, glutathione on different reactive electrophiles, many of which are potentially toxic and can bind to proteins and DNA resulting in cellular damage or genetic mutation (Josephy, 2010). These reactions generally detoxify the compound and facilitate their excretion in urine due to increased water solubility. Human GSTs can be divided into two main groups: membrane bound GSTs and cytosolic (soluble) GSTs (Mannervik et al., 2005).

Membrane-bound GST proteins are abundant in the

endoplasmic reticulum and the outer mitochondrial and account for about 4% of total protein in these membranes while cytosolic GSTs account for 10% of total cellular proteins and are divided into several classes on the basis of sequence similarity that reflects evolutionary branching from an ancestral protein (Mannervik et al., 2005).

Polymorphisms have been reported among the cytosolic GSTs, which are dimeric enzymes made up of homo- or hetero-dimers with each subunit in the dimeric enzyme being catalytically independent (Lai et al., 2005). There are at least eight documented classes of cytosolic mammalian GSTs with polymorphisms in humans been well established in three classes, GST μ (M1), GST θ (T1) and GST π (P1) (Board et al., 2000; Strange et al., 2001; Songbo et al., 2008). GSTM1 and GSTT1 both have a deletion polymorphism while GSTM1 and GSTP1 have alleles with sequence variations (Josephy, 2010).

The molecular basis of the lack of expression of

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GSTM1 was shown to be the result of a deletion of the whole GSTM1 gene, and this variant was designated as GSTM1*0 (Lai et al., 2005). Approximately, 50% of Caucasians are homozygous for the GSTM1*0 allele (Zhang et al., 1999). Two GSTT1 enzymes have been identified in man, GSTT1 and GSTT2 (Webb et al., 1996) and their genes are mapped to chromosome 22 (Lai et al., 2005). GSTT1 is very active in conjugation and detoxification of xenobiotics such as butadiene epoxide, bromodichloromethane, dichloromethane, ethylene dibromide, methylene chloride and ethylene oxide (Pemble et al., 1994; DeMarini et al., 1997). It displays significant activity in human erythrocyte and is thought to remove alkylating agents from circulation and prevent them from being transported to tissues where they could produce genotoxic damage. Nearly, 10-15% of Caucasians, 65% of Orientals and 10% of Mexicans do not express the GSTT1 gene (Katoh et al., 1996; Cotton et al., 2000; Sarmanova et al., 2000; Hlett et al., 2000). The biological consequences of the homozygous GSTT1*0 are difficult to predict, since this enzyme has both detoxifying and toxifying properties, depending on the nature of the substrate. For example, it has been reported that GSTT1 will detoxify monohaloalkanes but will toxify dihaloalkanes to reactive intermediates (Josephy, 2010).

GSTP1 is a major enzyme involved in the inactivation of cigarette smoke carcinogens such as benzo[a]pyrene diol epoxide and other toxic cigarette smoke constituents, such as acrolein (Fields et al., 1998). The majority of human tumours and human cancer cell lines over-express GSTP1 (Board et al., 2000) although, in some cases, such as in prostate carcinoma tissue, GSTP1 levels are down-regulated (Ntais et al., 2005). The exact significance of GSTP1 over-expression is poorly understood. The molecular mechanisms for polymorphism in the GSTP1 class are two point mutations on the GSTP1 gene. The two transitions are characterized by a change A³¹³>G³¹³ and a C³⁴¹>T³⁴¹ that result in amino acid changes Ile¹⁰⁵/Val¹⁰⁵ and Ala¹¹⁴/Val¹¹⁴ respectively (Watson et al., 1998; Fryer et al., 2000).

Members of the GST supergene family have linkage to a number of disease conditions such as cancers and asthma, as enzymes encoded by members of the GST gene families are critical in the protection of cells from reactive oxygen species (ROS) because they utilize a wide variety of products of oxidative stress as substrates (Cotton et al., 2000). Thus, the enzymes encoded by these GST gene classes preferentially utilize different ROS products. For example, quinone metabolites of catecholamines (dopachrome) are utilized by GSTM1 and GSTT1 utilizes oxidized lipid and DNA while GSTP1 catalyzes the detoxification of base propanals that arise from DNA oxidation. The general consensus from previous studies indicates that the GSTM1*0 allele may be an important risk factor for lung, colorectal and bladder cancer among smokers while homozygous GSTT1*0 may reduce risks associated with urothelial

cancer (Katoh et al., 1998). Considerations of combinations of GST polymorphic variants with other important drug metabolizing enzyme genotypes may prove important instead of looking at a single gene enzyme variant in searching for susceptible genes.

Nigeria is a multiethnic society with the largest population in the African continent and a home land to at least 250 languages (Robinson, 2004; <http://en.wikipedia.org/wiki/Nigeria>) but it is comprised mainly of three major ethnic groups namely; Hausa, Ibo and Yoruba, which together constitute over 65% of the Nigerian populations (<http://www.population.gov.ng>) (2006). In spite of the great ethnic variability associated with GSTs as well as its importance in xenobiotics detoxification, its polymorphisms have rarely been studied in Nigerian populations, which informed this present study. The objective of the present study was to perform a genetic analysis of GSTM1 and GSTT1 using a polymerase chain reaction (PCR) technique, in order to determine the frequency of the null genotypes in the Hausa, Ibo and Yoruba ethnic populations of Nigeria.

MATERIALS AND METHODS

Subjects

Three hundred healthy, unrelated subjects consisting of 215 males and 85 females, aged 18 to 45 years, who met the study inclusion criteria, were randomly selected, from the three major Nigerian ethnic groups of Hausa, Northern region (N=98), Ibo, Eastern region (N=101) and Yoruba, Western region (N=101) of Nigeria. Details of the study procedures were explained to the potential subjects after which, they were given an opportunity to make an independent decision to participate in the study. Eligible subjects were enrolled after signing the consent form and were classified as belonging to a particular ethnic group based on family history up to two previous generations. The ethics committee of Obafemi Awolowo University Teaching Hospital, Ile-Ife, approved the study.

Genomic DNA preparation

Qualified personnel withdrew 5 ml of blood sample from each participant using a syringe by veno-puncture into labelled EDTA tubes. After collection, the blood samples were frozen at -20°C until further analysis. The DNA was prepared using the QIAamp DNA blood mini kits (Qiagen, Belgium). Each frozen blood sample was thawed at room temperature and transferred to a clean polypropylene tube after pre-purification. Four (4) sets of tubes were labelled for each sample using a convenient labelling system and each set was arranged on a separate tube rack. For the third sets of tube a column was added while for the fourth sets a secure label was made by adding sellotape on label (tubes and columns were provided in the kit). After cell lysis cellular proteins were precipitated by salt precipitation leaving the high molecular weight genomic DNA in solution, which was concentrated and desalted by isopropanol precipitation. The DNA was bound to a column/resin washed using concentrated ethanol and eluted or dissolved in a low salt solution. The quality and the quantity of DNA was determined using UV spectrophotometer (Shimadzu, Japan). DNA samples were then stored at 4°C prior to genotyping analysis and aliquot of the sample was stored at -20°C for long-term use.

Genotyping

Genotyping for the GSTM1 polymorphism was performed by polymerase chain reaction (PCR) in accordance to the method of Brockmoller et al. (1992) and the GSTT1 null genotype was determined as described by Pemble et al. (1994). The PCR reaction was carried out on a programmable thermal cycler (LongGene Scientific Instruments). Primers complimentary to the GSTM1, GSTT1 and β -Actin control primers were used and their sequences as well as the PCR conditions are as shown in Table 1. The PCR incubation mixture constituents in a total volume of 25 μ L were: 200-300 ng of genomic DNA (1-2 μ L), 0.25 μ M of each primer, 0.2 mM of each deoxynucleotide triphosphate, 0.625 units of Taq polymerase and 2.5 μ L of x10 PCR buffer. The PCR products were visualized on 2% agarose gel electrophoresis by loading of the amplified product on the gel while 10 μ L of the diluted molecular weight marker was loaded to the gel for analysis. The gel was ran at 100 V, allowing migration of 2.5-3 cm. The result of the gel was viewed using a gel photo system GFS1000 (Fran Techtum Lab, Sweden).

Statistical analysis

The GSTM1 and GSTT1 genotype frequencies were obtained by direct counting and their relationship among the populations were determined by the odd ratio (OR) and 95% confidence interval (CI) using student's t-test of the SPSS statistical software with $p < 0.05$ as the level of significance.

RESULTS

The PCR assay typical of GSTM1 is as shown in Figure 1. The gel picture shows that β -actin 1 and β -actin 2 were added as Internal primers to the PCR reaction mix to act as control. The presence of β -actin fragment was shown by a band of 600 bp while the presence of the 273 bp fragment identifies a GSTM1 fragment. The absence of the 273 bp fragment in the presence of β -actin fragment indicates GSTM1 gene deletion (GSTM1*0). The subjects NGH5, 6, 8, 9 and 10 were likely homozygous or heterozygous to the wild-type, GSTM1*1 while NGH1 was homozygous to the null genotype, GSTM1*0.

Table 2 shows the results of GSTM1 and GSTT1 genotyping and the frequencies of combinations of genotypes for GST among the three ethnic groups. The homozygous null GSTM1*0 genotype was found to be 37% in Hausa, 23% in Ibo and 31% in Yoruba. The homozygous GSTT1*0 genotype was found at comparable frequencies of 42, 36, and 35% in the Hausa, Ibo and Yoruba populations, respectively. On summation, 112/300 (37%) were homozygous for GSTT1*0. On the average, 13% of the participants were homozygous for both GSTM1*0 and GSTT1*0.

DISCUSSION

The deletion polymorphisms of GSTM1 and GSTT1 were investigated by genotyping. The frequencies of the GSTM1 and GSTT1 genes were calculated only by

comparing the homozygous deletion of the genes because the determination of heterozygosity in individuals carrying the GSTM1 and GSTT1 genes were however not practicable as the 273 and 600 bp fragments, respectively, signifying the presence of the two genes could still be seen.

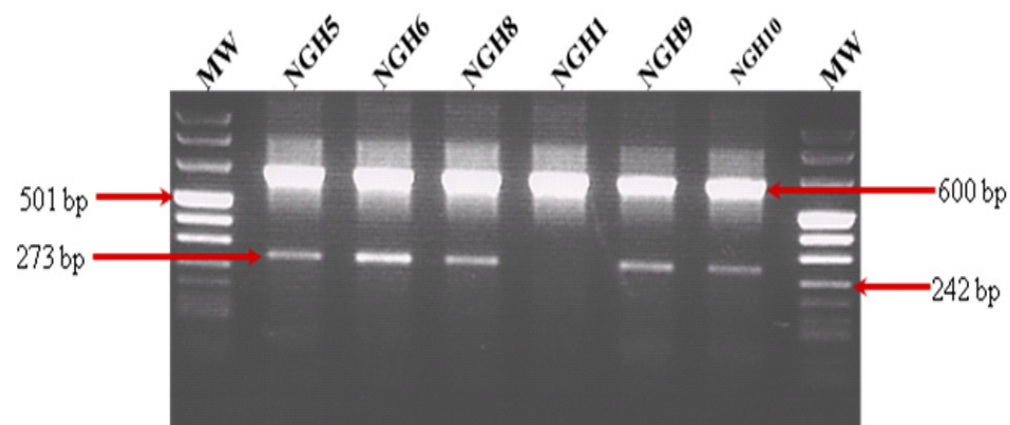
The homozygous null GSTM1*0 genotype was found to be 37% in Hausa, 23% in Ibo and 31% in Yoruba. There was no significant difference ($P > 0.05$) in the frequency of the homozygous GSTM1*0 in the Hausa and Yoruba. However, there was significant difference ($P < 0.05$) in the frequency of the GSTM1*0 in the Ibo relative to the Hausa and Yoruba populations. The findings of relatively low homozygous GSTM1*0 mean frequency of 30% in the three major Nigerian ethnic groups is in agreement with the 27% frequency reported for eastern and southern African populations (Dandara et al., 2002), African-Americans of 35 and 28% (London et al., 1995; Chen et al., 1996). This is comparable to the 22% phenotype frequency reported on Nigerians (Zhao et al., 1994) but lower than those reported among Caucasians, 40-60% and Asians, 55-80% (Rebeck et al., 1999; Persson et al., 1999; Lai et al., 2005). The slight variation in frequencies between this study and the previous one may be due to the fact that this study involved a more comprehensive sampling of all the major ethnic groups in Nigeria as well as the advantage genotyping offers in which case the genetic status of an individual does not change over time unlike phenotyping, where factors such as, environment, diet and age could influence it. The clinical implication of the findings is such that since the GSTM1 enzyme is thought to play a role in cancer prevention (Ntais et al., 2005), then the Nigerian population and indeed Africans in general will be better protected than their Asians and Caucasians counterparts from the effects of reactive metabolites such as, benzo(a)pyrene, known to cause lung cancer because of the lower frequency of GSTM1 gene deletion.

The homozygous GSTT1*0 genotype was found at comparable frequencies of 42, 36, and 35% for the Hausa, Ibo and Yoruba population, respectively. On the average 37% were homozygous for GSTT1*0 and 13% had both homozygous GSTM1*0 and homozygous GSTT1*0. The frequency of homozygous GSTT1*0 genotype in the three major Nigerian ethnic groups was higher than the frequency of 20-26% reported in South African Vendas, Tanzanians, Zimbabweans and African-Americans (Cotton et al., 2000; Dandara et al., 2002) and also higher than in Caucasians (13-18%) but lower than the 58% in the Chinese (Krajcinovic et al., 1999; Lai et al., 2005).

It is well known that GSTT1 is involved in the bioactivation of carcinogens, such as dihalogenated solvents acting in bladder carcinogenesis, thus populations with high frequencies of homozygous null GSTT1*0, such as the Chinese and most likely the Nigerian populations as seen in this study, may be better

Table 1. Primers sequence and PCR conditions for GSTM1 and GSTT1 genotyping.

Gene (allele)	Primers sequence	PCR conditions	Agarose gel fragment pattern
GSTM1*0 (homozygous deletion)	F 5'-CTGCCCTACTTGATTGATGGG-3' R 5'-CTGGATTGTAGCAGATCATGC-3'	35 cycles of 94 °C at 20 sec, 53 °C at 30 sec, 72 °C, 30 sec	273 bp for GSTM1
B-AcH control primers	F 5'-TGACGGGGTCACCCACACTGTGCCCATCTA-3' R 5'-TAGAAGCATTGCGGTGGACGATGGAGGG-3'	35 cycles of 94 °C at 20 sec, 53 °C at 30 sec, 72 °C at 30 sec	600 bp for batch control
GSTT1*0	F 5'-TTCCTTACTGGTCCTCACATCTC-3' R 5'-TCACCGGATCATGGCCAGCA-3'	35 cycles of 94 °C at 20 sec, 56 °C at 30 sec, 72 °C at 30 sec	600 bp for GSTT1

**Figure 1.** The PCR detection of the GSTM1*0 genotype carried out on a 2% agarose gel. MW: molecular weight marker. NGH5, 6, 8, 9 and 10: homozygous or heterozygous GSTM1*1; NGH1: homozygous GSTM1*0.**Table 2.** Frequency distribution of GST genotypes in the Hausa, Ibo and Yoruba ethnic populations of Nigeria.

Homozygous genotypes	Hausa N/total (%)	Ibo N/total (%)	Yoruba N/total (%)	Mean N/total (%)
GSTM1*0/ GSTM1*0	36/98 (37)	23/101 (23)	31/101 (31)	90/300 (30)
GSTT1*0/ GSTT1*0	41/98 (42)	36/101 (36)	35/101 (35)	112/300 (37)
GSTM1/GSTT1	36/98 (37)	50/101 (50)	47/101 (47)	133/300 (44)
GSTM1*0/GSTT1	18/98 (18)	14/101 (14)	17/101 (17)	49/300 (16)
GSTM1/GSTT1*0	23/98 (23)	26/101 (26)	22/101 (22)	71/300 (24)
GSTM1*0/GSTT1*0	17/98 (17)	9/101 (9)	13/101 (13)	39/300 (13)

protected against bladder cancer initiation than the Caucasians with reported low frequency of the GSTT1 deletion. Basically, GST enzymes play a key role in detoxification processes in the body. However, some pathways catalyzed by GST enzymes can lead to more toxic products, therefore, expression of GSTM1 and GSTT1 and exposure to certain substrates might pose risks rather than advantage. For instance, Methylchloride is converted to the more toxic formaldehyde (Josephy, 2010). The deleterious effects as well as the advantages of GST polymorphisms could be responsible why nature maintains the null phenotypes in populations. The finding in this study shows that the genotype frequencies of the null GSTM1 and GSTT1 were relatively high in Nigerian populations with a possible implication on some cancers susceptibility.

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