

RESEARCH COMMUNICATION

Allelic Variation of GSTT1, GSTM1 and GSTP1 Genes in North Indian Population

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Abstract

Glutathione S-transferase (GST) enzymes are involved in detoxification of many potentially carcinogenic compounds. Homozygous deletions or null genotypes of GSTT1 and GSTM1 genes and an A to G substitution at nucleotide 313 in GSTP1 have been reported in different populations. Intra-ethnic as well as interethnic differences are known to exist in the frequencies of the above GST genes. The present study was therefore undertaken to determine the prevalence of GSTM1 and GSTT1 null alleles, as well as the GSTP1 gene polymorphism, in 370 healthy individuals in a North Indian population. Genotyping of M1 and T1 was performed using a multiplex polymerase chain reaction and the GSTP1 polymorphism was determined by the polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) method. The frequencies of GSTM1 and GSTT1 null alleles in normal healthy individuals were observed to be 33.0% and 18.4% respectively. In 7.0% of individuals' concomitant lack of M1 and T1 genes were observed. For GSTP1, wild (Ile/Ile), heterozygous (Ile/Val) and mutant (Val/Val) genotypes were observed for 44.3%, 50.3% and 5.4% of individuals respectively. The prevalence of the M1 null allele is significantly lower than those documented for English, Turkish, Chinese, Caucasians, Japanese and white (Brazilian and American) populations. However, a significantly higher frequency for T1 null was reported in Chinese and Japanese population. Furthermore, Japanese and African American populations have exhibited significantly higher frequencies of wild and mutant P1 genotypes, respectively, than the Indian population. Thus, our results signify an impact of ethnicity and provide a basis for future epidemiological and clinical studies.

Key Words: GSTM1 - GSTT1 - GSTP1 - North India - Polymorphism

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Introduction

Epidemiological evidence indicates that most of human cancers are originally caused by environmental exposure to genotoxic agents. In order to protect cells from DNA damage by various forms of reactive substances, a cellular system for detoxification has evolved.

Glutathione transferases (GSTs) are complex multigenic family of phase II metabolizing enzymes that are responsible for many biological functions like detoxification of a large number of electrophiles. The resultant conjugation reactions lead to synthesis of mercapturic acids and represent an important excretory route for many xenobiotics (Mannervik, 1985). GSTs of the mu class catalyse the detoxification of genotoxins including aromatic hydrocarbon epoxides and products of oxidative stress such as DNA hydroperoxides (Hcagerty et al., 1994). The GSTT1 class utilizes constituents of cigarette smoke such as alkyl halides (Pemble et al., 1994) while GSTP1 class enzymes target cigarette smoke derived chemicals such as benzo (a) pyrene diol epoxide and acrolein (Hayes and Pulford, 1995). Examples of allelic variation

have been identified in GSTM1, GSTT1 and GSTP1, associated with an increased risk of cancer.

For GSTM1 and GSTT1, variants include homozygous deletion of the gene or a null genotype (Pemble et al., 1994), and the prevalence has been found to vary among ethnic groups (Nelson et al., 1995; Kimyohara, 2000). About 35-60% of individuals (Katoh et al., 1996; Chenevix-Trench et al., 1995; Bell et al. 1993) and 10-65% (Nelson et al., 1995, Chenevix-Trench et al., 1995) have been reported to possess null genotypes for GSTM1 and GSTT1 respectively.

Numerous studies have revealed that GSTM1 and GSTT1 null genotypes increase susceptibility to lung cancer (Kiyohara et al., 2000), bladder cancer (Kempkes et al., 1996), gastric cancer (Sctiawan et al., 2000) and colorectal cancer (Welfare et al., 1999) either individually or in combination.

Two genetic polymorphisms have been identified in GSTP1 gene that are associated with reduced enzymatic activity and altered enzyme kinetics. In one the P1 variant allele contains an A313G change, leading to an Ile to Val substitution in amino acid 105 within the active site of the

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enzyme. The other P1 variant allele features an Ala to Val change at amino acid 114 (Ali-Osmam et al., 1997).

Since allelic and genotypic variations have been observed in different populations and ethnic groups in various parts of world, we here define the allelic profiles and frequencies for GSTM1, T1 and P1 in healthy individuals from North India, for comparison with data from elsewhere.

Materials and Methods

DNA Isolation

Blood samples were randomly collected in sterile 0.5M EDTA vials from 370 normal healthy unrelated individual (Age range 30-85 years) from North Indian population after informed consent. The inclusion criterion for the controls was absence of prior history of cancer or any other related disease. DNA was extracted from whole blood using the standard phenol-chloroform method (Sambrook et al., 1989).

M1 and T1 Genotyping

Multiplex PCR was performed as described by Abdel-Rahman et al (1998) to determine the presence or absence of GSTM1 and GSTT1 genes. CYP1A1 was co-amplified and used as an internal standard. 100ng of DNA was amplified in a total volume of 25µl reaction mixture containing 10pmol of each primer as described (Table 1), 200µmol of dNTPs and 1.5U Taq polymerase (Gibco-BRL). PCR was performed with initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 1min, 59°C for 1min, 72°C for 1min and a final extension at 72°C for 10 min. The M1 and T1 genotypes yielded bands of 215bp and 480bp, respectively, with an internal control of 312bp (Fig1).

GSTP1 Genotyping

GSTP1 was identified by digestion of the PCR products using the PCR restriction fragment polymorphism method. (Harries et al., 1997) in a total volume of 25 µl using 10pmol each of forward and reverse primers (Table 1). Initial denaturation was carried out at 95°C for 5min followed by 30 cycles at 95°C for 2min, 55°C for 30sec, 72°C for 30sec and final extension at 72°C for 5min. 10µl of 176bp PCR product was digested for 2 hrs at 55°C with 5U of Alw261 (NEB, UK) and separated on a 3.5% ethidium bromide stained gel (Fig 2). With the A to G transition, Alw261 was cleaved, yielding two fragments of 91bp and 85bp.

Statistical Analysis

Allele and genotype frequencies of M1, T1 and P1 in

Table1. Oligonucleotide Primers Used for Amplification

GSTM1	F - 5' GAA CTC CCT GAA AAG CTA AAG C 3'
	R - 5' GTT GGG CTC AAA TAT ACG GTG G 3'
GSTT1	F - 5' TTC CTT ACT GGT CCT CAC ATC TC 3'
	R - 5' TCG CCG GAT CAT GGC CAG CA 3'
CYP1A1	F - 5' GAA CTG CCA CTT CAG CTG TCT 3'
	R - 5' CAGCTGCATTTGGAAGTGCTC 3'
GSTP1	F 5' ACC CCA GGG CTC GAT GGG AA 3'
	R 5' TGA GGG CAC AAG AAG CCC CT 3'

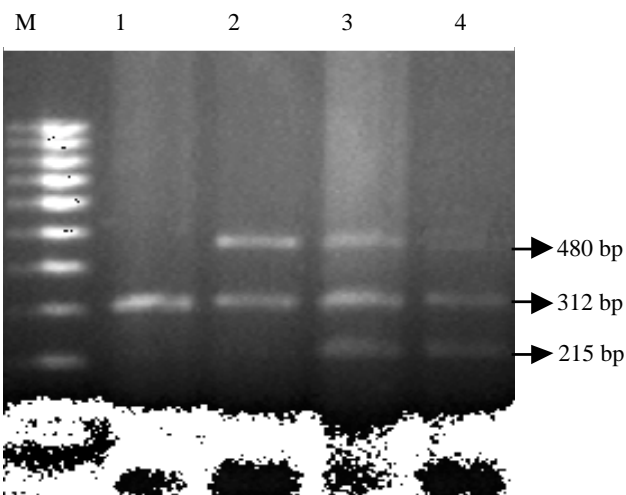


Figure 1. Gel Showing Different Allelic Conditions for the M1 and T1 Genes M – 100bp ladder; Lane -1 depicts M1 and T1 null alleles, Lane-2 is M1 positive; Lane-3 signifies M1 and T1 positive and Lane-4 is T1 positive

normal healthy controls in north Indian population were determined according to the Hardy –Weinberg equilibrium. The frequency distributions for M1, T1 and P1 alleles in different age ranges were determined by logistic regression. The Fisher's exact and chi square test were applied to compare the allelic frequencies in different populations using the SPSS (version 10.0) software programme for Windows.

Results

GSTM1 and T1: Data for the frequency distribution of GSTM1 and GSTT1 alleles in our north Indian population are presented in Table 2. The mean age of the subjects was 51.8 ± 11.3 . The GSTM1 null genotype was observed in 122 (33.0%) individuals. For GSTT1, 68 (18.4%) individuals were null type. Twenty-six (7%) showed a concurrent lack of M1 and T1 and 138 (37.02%) were positive for either of the genes. Using logistic regression, the age range distribution was compared with M1 or T1 alleles but there was no significant association ($p > 0.05$). The frequency distributions of M1 and T1 alleles in different populations and comparison with Indian population by Fisher exact test are presented in Table 3.

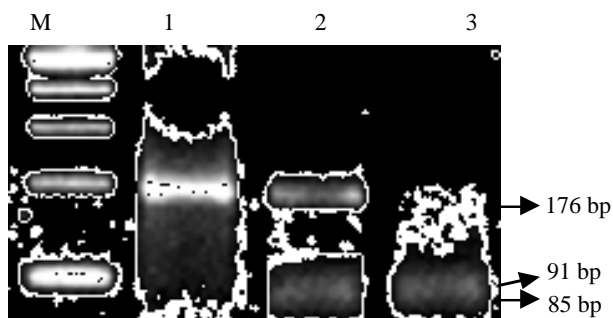


Figure 2. Gel Picture of GSTP1 PCR Products After Restriction Digestion

M – 100 bp ladder, Lane-1 shows Wild type (Ile/Ile); Lane-2 Heterozygous (Ile/Val) and Lane -3, Mutant type (Val/Val)

Table2. Frequency Distribution of GSTM1 and GSTT1 Alleles in the North Indian Population

N= 370	GSTM1 genotype		GSTT1 genotype		GSTM1+ GSTT1	
	Frequency %		Frequency %		Frequency %	
Absence (null)	122	33	68	18.4	26	7
Presence	248	67	302	81.6	344	93

GSTP1: The overall frequencies of GSTP1 alleles and genotypes are shown in Table 4. The P1 genotype distribution was in Hardy- Weinberg equilibrium. The prevalence for the wild type Ile homozygous variant was 164 (44.3%), for the heterozygous Ile/Val variant was 186 (50.3%) and for the homozygous Val/Val variant was 20 (5.4%). Logistic regression analysis showed no association between the age distribution and GSTP1 genotypes. The Ile allele was dominant in 69.5% individuals.

Comparing GSTP1 genotypes with M1 and T1 null alleles, 0.8% were mutant for P1 and M1, 3.5% were mutant for P1 and T1 (p<0.01) and 0.8% were mutant for all three genes.

In order to compare the allele and genotype frequency distribution with other populations, the chi-square test was employed (Table 5).

Discussion

The molecular epidemiology of cancer involves the use of biomarkers of exposure and response of exogenous or endogenous agents and/or host factors that play a role in etiology. Variation in the expression of GSTs due to genetic polymorphism probably modulates the process of carcinogenesis by altering the exposure levels to active carcinogen metabolites. Identification of these SNPs (single nucleotide polymorphisms) in the human genome has great implications for studies of disease susceptibility. The Indian population is believed to be most diverse because of different socio-cultural traditions. The present GST genotyping study was based on molecular methods for analyzing the frequency distribution in north Indian normal controls.

Our observations for GSTM1 are similar to the findings reported for a south Indian population (Naveen et al., 2004). But the frequency of GSTM1 null individuals obtained in our study was significantly lower (33%) than other populations (Table 3). Similarly, the GSTT1 null genotype was observed in 18.4% individuals, in agreement with a study conducted by Naveen et al., (2004) in south Indians , and in other populations also (Table 3). Only Chinese (Sctiawan et al., 2000) and Japanese (Kiyohara et al., 2000) demonstrated a significantly (p< 0.01) higher frequency of GSTT1 null alleles. The frequency distribution of GSTP1 genotypes in our study is also in agreement with other reports for different populations (p>0.05, Table 5). Only Japanese (Kiyohara et al., 2000) and African Americans appear to exhibit a significant difference (p<0.05) in frequency distribution. Japanese have a complete absence of the mutant homozygous (Val/Val) genotype while in African Americans mutant type (Val) allele dominates. These findings may be accredited to ethnic differences in the frequency of the M1 and T1 null genotypes.

We also studied individuals with concurrent lack of M1, T1 and the P1 standard type allele (Ile). It revealed that only 0.8% of individuals demonstrated higher susceptibility to related diseases (absence of M1, T1 and P1 wild allele).

The variation in our Indian population from the rest of the world population signifies the impact of ethnicity. Thus, this kind of study may form the basis for future establishment of epidemiological and clinical databases. This approach also has the potential for identifying susceptible individuals. The present work thus provides probably the first study of this nature from India. We believe that further investigation of GSTM1, GSTT1 and GSTP1 allelic variants in north Indians should provide useful information for identification of founder mutations and ethnic predisposition alleles that result in various cancerous disease phenotypes.

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Table 3. Comparative Frequency Distribution of GSTM1 and GSTT1 Alleles in Various Populations

Country/ Population	No (N)	GSTM1 (%)		Deviation (p)	GSTT1 (%)		Deviation (p)	Reference
		Null	Presence		Null	Presence		
North India	370	33	67	Ref.	18.4	81.6	Ref.	(Present study)
Newcastle, England	178	50.8	49.2	0.01	16.9	83.1	1.00	Welfare et al, 1999
Central Europe	127	45	55	0.11	13	87	0.43	Steinhoff C et al, 2000
Turkish	133	51.9	48.1	0.01	17.3	83.7	1.00	Ada et al, 2004
Italians	273	46.9	53.1	0.06	19	81	1.00	D'Alo et al, 2004
South India	517	30.4	69.6	0.76	16.8	83.2	1.00	Naveen et al, 2004
Chinese	477	51	49	0.01	46	54	<0.01	Sctiawan et al, 2000
Caucasian	166	48.8	51.2	0.03	19.9	80.1	0.86	Gsur et al, 2001
Japanese	88	55.7	44.3	<0.01	44.3	55.7	<0.01	Kiyohara et al, 2000
Finnish Caucasians	478	41.8	58.2	0.24	13.2	86.8	0.43	Mitrunen et al, 2001
African Americans	271	28	72	0.54	17	83	1.00	Millikan et al, 2000
Whites (USA)	392	52	48	0.01	16	84	0.85	Millikan et al, 2000
Brazilian non Whites	272	34.2	65.8	1.00	25.7	74.3	0.23	Rossini et al, 2002
Brazilian Whites	319	48.9	51.1	0.02	25.1	74.9	0.30	Rossini et al, 2002

Table 4. Frequency Distribution of GSTP1 Genotypes and M1, T1 Null Alleles in North Indian Population

N=370	Frequency (%)	GSTM1 Null	GSTT1 Null	M1 and T1 Null
Standard (Ile/Ile)	164 (44.3)	57	31	13
Hetero (Ile/Val)	186 (50.3)	62	24	10
Mutant (Val/Val)	20 (5.4)	3	13	3

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Table 5. Comparative Frequency Distribution of GSTP1 Alleles in Various Populations

Country/ Population	No (N)	Genotype (%)			Deviation (p)	Alleles (%)		Deviation (p)	References
		Ile/Ile	Ile/Val	Val/Val		Ile	Val		
North India	370	44.3	50.3	5.4	Ref.	69.5	30.5	Ref.	Present study
Newcastle England	178	44.9	43.4	11.7	0.18	66.6	33.4	0.76	Welfare et al, 1999
Caucasian	166	39.2	47.3	13.3	0.13	63	37	0.37	Gsur et al, 2001
Japanese	88	70.5	29.5	0	<0.00	85.3	14.7	0.02	Kiyohara et al, 2000
Finnish Caucasians	481	55.3	37.6	7.1	0.23	74.1	25.9	0.64	Mitrunen et al, 2001
African Americans	271	22	55	23	<0.00	49.5	50.5	0.006	Millikan et al, 2000
White (USA)	392	40	49	11	0.28	64.5	35.5	0.55	Millikan et al, 2000
Brazilian Non White	272	47.8	42.6	9.6	0.36	69.1	30.9	1.00	Rossini et al, 2002
Brazilian White	319	51.4	34.2	14.4	0.20	68.5	31.5	1.00	Rossini et al, 2002