RESEARCH COMMUNICATION

Genetic Variation of GSTM1, GSTT1 and GSTP1 Genes in a South Indian Population

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Abstract

The glutathione S transferase (GST) family of enzymes play a vital role in the phase II biotransformation of environmental carcinogens, pollutants, drugs and other xenobiotics. GSTs are polymorphic and the polymorphisms in GST genes have been associated with cancer susceptibility and prognosis. Moreover, distinct ethnic differences have been observed in the type and frequency of GST gene polymorphisms. Hence, the present study was aimed to determine the frequencies of GSTM1, GSTT1 and GSTP1 polymorphisms in 255 healthy random volunteers from South India. The GSTM1 and GSTT1 genotypes were determined by PCR and GSTP1 by PCR-RFLP using peripheral blood DNA.The GSTM1 and GSTT1 null genotype frequencies were found to be 22.4% and 17.6% respectively. The GSTP1 allelic frequency was 0.78 for the Ile allele and 0.22 for the Val allele and the genotype frequency was 58.4% for Ile/Ile, 38.4% for Ile/Val, and 3.1% for Val/Val. Comparison of the frequencies of GST polymorphisms observed in the present study with other Indian and world populations revealed a distinctive nature of the South Indian population with respect to polymorphims at the GST gene loci. A better understanding of carcinogen metabolizing gene distribution should contribute to risk assessment of humans exposed to environmental carcinogens.

Key Words: GSTM1 - GSTT1 - GSTP1 - polymorphisms

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Introduction

The glutathione S-transferases (GST), a superfamily of phase II metabolic enzymes play an important role in the cellular mechanism of detoxification by conjugating reactive electrophilic compounds with soluble glutathione (Strange et al., 1998). GST enzymes are thus involved in the metabolism of xenobiotics that include environmental carcinogens, reactive oxygen species and chemotherapeutic agents (Hayes and Strange, 2000). A large number of structurally diverse xenobiotics are known to be substrates for the GSTs. Some substrates have relatively high specific activity with one or a few isoenzymes within a class and little or no activity within other classes (Whalen and Boyer, 1998). In addition, these enzymes are also believed to play a crucial role in the protection of DNA from oxidative damage.

At least eight distinct classes of soluble GSTs that are highly expressed in the mammalian liver have been identified, which include alpha, mu, pi, sigma, theta, kappa, zeta and omega. In humans, hereditary differences in some GST enzyme activities are due to genetic polymorphisms. Polymorphism has been described in many genes in these families though to date, more attention has been focused on alleles in the mu, theta and pi families (Hayes and Strange, 2000).

The known substrates for GSTM1 include metabolically generated epoxide intermediates of benzo [a] pyrene and other polyaromatic hydrocarbons, whereas the substrates for GSTT1 include alkyl halides found in cigarette smoke and lipid peroxides (Hayes et al., 1995). The null genotypes that are associated with a lack of enzyme function exist at both these loci. GSTP1 is a major enzyme involved in the inactivation of cigarette smoke carcinogens, such as benzo [a] pyrene diol epoxide, and other toxic constituents, such as acrolein (Hayes, 1995). GSTP1 has a polymorphic site at codon 105 (exon 5), where an adenosine-to-guanosine (A-G) transition at nucleotide 313 results in an Ile-to-Val substitution (Ile105Val) in the substrate-binding site of GSTP1. The substitution of the less bulkier and more hydrophobic valine results in substrate-dependent alterations of GSTP1 catalytic activity (Ali-Osman et al., 1997, Sundberg et al., 1998).

Since the polymorphisms of these metabolizing genes influence the detoxifying action, they have been suggested to play an important role in cancer susceptibility and prognosis. Allelic variants of GSTM1, GSTT1, and GSTP1 have been associated with increased risk of various cancers like colorectal, lung, breast, prostate and others (Kimyohara et al., 2000; Kiyohara et al., 2000; Mitrunen et al., 2001,

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Table 1. Oligonucleotide Primers for PCR

Gene	Primer sequence	Size
GSTM1		
	Forward 5'-ACTCCCTGAAAAGCTAAAGC-3'	220 bp
	Reverse 5'-GTTGGGCTCAAATATACGGTGG-3'	
GSTT1		
	Forward 5'-TTCCTTACTGGTCCTCACATCTC–3'	450 bp
	Reverse 5'-TCACCGGATCATGGCCAGCA-3'	
Intron 3 of HLADRB1		
	Forward 5'-TGCCAAGTGGAGCACCCAA-3'	796 bp
	Reverse 5'-GCATCTTGCTCTGTGCAGAT-3'	
GSTP1		
	Forward 5'-CCAGGCTGGGGCTCACAGACAGC-3'	306 bp
	Reverse 5'-GGTCAGCCCAAGCCACCTGAGG-3'	

Vijayalakshmi et al., 2005). The allelic and genotypic variations have been observed in different populations and ethnic groups in various parts of the world. Absence of GSTM1 activity has been reported in 40% to 60% of the Caucasians as a result of the inheritance of two null alleles, along with absence of GSTT1 activity in 20% to 30% of Caucasians (Rebbeck et al., 1997). As, India is known for its unique population structure; having about 5000 endogamous populations we here define the allelic profiles and frequencies for GSTM1, GSTT1 and GSTP1 in healthy random unrelated individuals from South India.

Materials and Methods

Subjects

The study population comprised of 255 random healthy unrelated individuals from South India. Of the 255 individuals, 91 were female and 164 were male. The age of the individuals ranged from 20-65years with mean age of 46 years. All the individuals were of South Indian ethnicity. Blood samples were collected from these individuals with informed written consent.

DNA Extraction and Genotyping

Genomic DNA was isolated from whole blood by the salting out method (Miller et al.1988). Genotypes of GSTM1 and GSTT1 were determined by multiplex PCR where the GSTM1 and GSTT1 genes were co amplified with the intron 3 of HLA DRB1 as internal control. The primers used for the analysis are given in Table 1. PCR reaction was performed in a total 20µl reaction volume containing 50-100ng of genomic DNA, 1X PCR buffer (1.5mM MgCl2, 10 mM Tris (pH 9.0), 50 mM KCl and 0.1% Triton X-100), 200µM dNTPs, 50pM of each primer and 1 U of Taq DNA polymerase. The cycling condition was 95º C for 5min of one cycle; 95º C for 1min, 60º C for 1min and 72º C for 1min 30sec for 30 cycles and final elongation cycle of 72ºC for 5min. The PCR products were visualized by 2% agarose electrophoresis and the genotype was determined by the presence or absence of 220bp PCR amplicon of GSTM1 gene and 450 bp of GSTT1 gene.

The GSTP1 Ile/Val polymorphism was determined by PCR followed by restriction fragment length polymorphism (PCR-RFLP) (Vijayalakshmi et al. 2005) the primers used for the analysis are given in Table 1. The cycling conditions were 94º C for 5min of one cycle; 94º C for 45sec, 66º C for 45sec and 72º C for 1min for 30 cycles and final elongation cycle of 72ºC for 5min.The PCR amplicon of 306bp was subjected to restriction digestion using HpyCHIV enzyme (New England Biolabs, Inc., USA) at 37˚C for 1hour and the DNA bands were resolved by electrophoresis on a 3% agarose gel. The genotypes were determined based on the band pattern. The Ile allele was resistant to digestion by HpyCHIV and so the Ile/Ile genotype resulted in an undigested band of 306 bp, the Val allele with HpyCHIV site resulted in two fragments of 183bp and 123bp. So an Ile/Val genotype was characterized by three fragments of 306bp, 183 bp and 123 bp and a Val/ Val genotype by two fragments of 183 bp and 123 bp.

Statistical Analysis

The distribution of the allele and genotype frequencies of GSTM1, GSTT1 and GSTP1 were determined. Chi square test was applied to compare the allelic frequencies in different population using the SPSS (version 13) software programme for windows.

Results

The genotype frequency distribution of GSTM1 and GSTT1 are shown in Table 2.The GSTM1 and GSTT1 null genotype was observed in 22.4% and 17.6% of the individuals respectively. Analysis of the combined distribution of the GSTM1 and GSTT1 genes revealed 4.3% of the individuals to exhibit a lack of both the genes, whereas, 64.3% were positive for both the genes.

For the GSTP1 genotype, 58.4% were homozygous for the wild type allele Ile/Ile, 38.4% were heterozygous Ile/Val and 3.1% were homozygous variant Val/Val. The allele frequency for Ile is 0.78 and Val is 0.22. The GSTP1 genotype distribution follows Hardy Weinberg equilibrium. Stratified analysis of the GSTP1 variation based on sex revealed that the wild type Ile/Ile was significantly higher in females (71.4%) than males (51.2%) and that the

Table 2. Distribution of GSTM1, GSTT1 and GSTP1 Genotypes and Allele Frequencies (n=255)

GSTM1		GSTT1			GSTP1						Allele frequency		_2	P
Presence	Null	Presence	Null		I/I	%	I/V	%	V/V	%	I	V		
198	57	210	45	Obs*	149	58.4	98	38.4	8	3.1	0.78	0.22	2.70	NS
(77.6%)	(22.4%)	(82.4%)	(17.6%)	Exp*	155		88		12					

Obs, Observed; Exp, Expected; NS, Non significant

Table 3. Distribution of combined genotypes of GSTP1, GSTM1 and GSTT1

GSTP1			GSTT1		Total
			Null	Presence	
I/I	GSTM1	Null	5	27	32
		Presence	20	97	117
		Total	25	124	149
I/V	GSTM1	Null	6	17	23
		Presence	13	62	75
		Total	19	79	98
V/V	GSTM1	Null	0	2	2
		Presence	1	5	6
		Total	1	7	8

heterozygous Ile/Val was significantly higher in males (45.1%) than in females (26.4%) (P<0.01). However, GSTM1 and GSTT1 variants did not exhibit significant difference between males and females.

Comparison of GSTP1 genotypes with GSTM1 and GSTT1 alleles is presented in Table 3. The allelic frequency distribution of GSTM1, GSTT1, and GSTP1 in different populations and comparison with the present study are represented in Table 4.

Discussion

Metabolic pathways of xenobiotics include their activation during phase I of the biotransformation process followed by conjugation of highly toxic intermediate metabolic products during phase II. Therefore, expression of phase I and II enzymes must be well coordinated. The genes encoding metabolizing enzymes are highly polymorphic, so the presence of variant alleles can provoke imbalanced interactions of phase I and II enzymes. The fact that high frequencies of the variant GST genotypes have been found in patients with environmentally induced cancers, GST genotype detection demands special attention. Moreover there is distinct ethnic variation in the GST genotype distribution and also in their cancer susceptibility and outcome. Hence the present study was performed to determine the GST genotype distribution among the ethnic South Indians.

The frequency of GSTM1 null genotype observed in the present study is significantly lower (22.4%) than the other populations (Table 4). Our observation on the GSTT1 null genotype is similar to other population except for the Japanese (Kiyohara et al., 2000) and the Chinese (Sctiawan et al., 2000) (Table 4). The frequency distribution of GSTP1 alleles observed in the present study is also significantly different from other populations (Table 4). The present study shows a distinct variation in the GSTM1 and GSTP1 frequencies compared to the North Indian population thus establishing the fact that there is a distinct difference among the Indian Population. This could be attributed to the fact that the South Indians are considered as the original inhabitants of Indian subcontinent and the North Indians are the migrant's population having a mixed gene pool (Coon 1983). This suggests that there exists inter ethnic variation in the frequency of polymorphic alleles at GST gene loci. These variations and also differences in disease susceptibility associated with the GST gene loci could be due to differences in the linkage or genetic associations between alleles in different population.

The polymorphic frequencies presented in our study can form a basis for identifying genetic risk factors associated with various environmentally induced disease phenotyes

Table 4. Comparative Frequency Distribution of GSTM1, GSTT1 and GSTP1 Alleles in Various Populations

	N	GSTM 1			GSTT1			GSTP				
		Null	Presence	P value	Null	Presence	P value	1Ile/Ile	Ile/val	val/val	P value	Reference
Present study	255	22.4	77.6	Ref	17.6	82.4	Ref	58.4	38.4	3.1	Ref	Present Study
North Indian	370	33.0	67.0	0.002	18.4	81.6	0.8154	44.3	50.3	5.4	0.002	Mishra et al 2004
English	178	50.8	49.2	<0.0001	16.9	83.1	0.8301	44.9	43.4	11.7	0.0003	Welfare et al, 1999
Central Europe	127	45.0	55.0	<0.0001	13.0	87.0	0.2873	NA	NA	NA	-	Steinhoff C et al, 2000
Turkish	133	51.9	48.1	<0.0001	17.3	83.7	0.9307	NA	NA	NA	-	Ada et al, 2004
Italians	273	46.9	53.1	<0.0001	19.0	81.0	0.6779	NA	NA	NA	-	DAlo et al, 2004
Chinese	477	51.0	49.0	<0.0001	46	54	<0.0001	NA	NA	NA	-	Sctiawan et al, 2000
Caucasian	166	48.8	51.2	<0.0001	19.9	80.1	0.5645	39.2	47.3	13.3	<0.0001	Gsur et al, 2001
Japanese	88	55.7	44.3	<0.0001	44.3	55.7	<0.0001	70.5	29.5	0.0	0.0571	Kiyohara et al, 2000
Finnish	478	41.8	58.2	<0.0001	13.2	86.8	0.1041	55.3	37.6	7.1	0.0893	Mitrunen et al, 2001
African-USA	271	28.0	72.0	0.13	17	83	0.8384	22.0	55.0	23.0	<0.0001	Millikan et al, 2000
Whites USA	392	52.0	48.0	<0.0001	16	84	0.5995	40.0	49.0	11.0	<0.0001	Millikan et al, 2000
Brazil Non-W	272	34.2	65.8	0.003	25.7	74.3	0.0247	47.8	42.6	9.6	0.0027	Rossini et al, 2002
Brazil White	319	48.9	51.1	<0.0001	25.1	74.9	0.0321	51.4	34.2	14.4	<0.0001	Rossini et al, 2002

NA- Data not available

among Indians and also for future establishment of epidemiological and clinical databases for identifying susceptible individuals. Moreover assessment of frequencies of polymorphisms at such gene loci can be used for prediction of the metabolic capacity of distinct ethnic groups and for designing individualized drug treatment.

In conclusion, our study provides an estimate of the frequencies of some of the polymorphic GST alleles in the South Indian population. The results indicate that the molecular profile of polymorphisms at the various GST loci is distinctly different among South Indians compared to other ethnic groups.

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