RESEARCH COMMUNICATION

Genetic Polymorphisms of *CYP2E1* and *GSTP1* in a South Indian Population - Comparison with North Indians, Caucasians and Chinese

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Abstract

CYP2E1 and GSTP1 enzymes belong to phase I and phase II group of drug metabolizing enzymes respectively which are involved in the metabolic activation and detoxification of various potential genotoxic compounds. The functional polymorphism in these genes exhibit inter-individual variations in susceptibility towards various diseases and difference in therapeutic response. The variant sequences of these genes differ considerably between ethnic groups. Therefore, the objective of the study was to assess the prevalence of *CYP2E1 & GSTP1* gene variants in healthy volunteers of Tamilnadu, a population of South India. The genotype distribution of *CYP2E1*1B A2A2, A2A1* and *A1A1* were 61%, 36% and 3% respectively. The distribution of *CYP2E1*5B c1c1, c1c2* genotypes were 99.2% and 0.8%. *CYP2E1*6 DD, DC* and *CC* genotype frequencies were 72%, 25% and 3% respectively. The allele frequencies of *CYP2E1*1B, CYP2E1*5B* and *CYP2E1*6* were *A2*- 0.79 *A1*- 0.21, *c1*-0.996 *c2* - 0.004 and *D*- 0.84 *C*- 0.16 respectively. The genotypic distribution of *GSTP1 (Ile/Val)* were *Ile/Ile* - 44%, *Ile/Val* -47% and *Val/Val*- 9 % whereas, the allelic frequencies were 0.67 for *Ile* and 0.33 for *Val* allele. The molecular studies in these enzymes provide basis for further epidemiological investigations in the population where the functional mutations in the genes alter therapeutic response and acts as susceptibility markers for various clinical conditions.

Key Words: Polymorphism - genotyping - Indian - Tamilian - *CYP2E1* - *GSTP1*

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Introduction

Chemical carcinogens and other xenobiotics can be activated or deactivated by phase I (cytochromes P-450) and phase II enzymes (glutathione S-transferases, NAT etc.). Polymorphisms in these detoxification enzymes lead to inter-individual variability for susceptibility to carcinogenesis.

CYP2E1 mapped in the region of 10q24, belongs to Cytochrome P450 (CYP) enzyme family. It represents a major CYP isoform in the liver (6%) and is also expressed at significant levels in human esophagus and other extrahepatic tissues (Tan et al., 2000; Correia, 2004). Among the Cytochrome P450s, the enzyme CYP2E1 is of particular interest because it is involved in the oxidation of ethanol to produce reactive free radicals that may initiate lipid peroxidation and subsequently liver injury (Kehrer, 1993; Albano et al., 1996). The enzyme is also induced in a variety of pathophysiological conditions like diabetes, obesity, cancer etc. (Caro and Cederbaum, 2004; Villeneuve and Pichette, 2004). It also participates in the metabolic activation of many low molecular carcinogenic and toxic chemicals, such as nitrosamines present in the tobacco smoke, vinyl chloride, styrene, carbon tetrachloride, ethylene glycol and benzene (Tanaka et al., 2000; Yu et al., 1995). Most studies report the association of non mutated alleles of CYP2E1 with the risk of developing cancers and liver diseases in humans (Gonzalez et al., 1998; Tan et al., 2000; Wong et al., 2000; Sobti et al., 2004). However, there are also a few studies where the mutant alleles have been linked to risk of various cancers (Uematsu et al., 1991; Hung et al., 1997).

So far about thirteen alleles of CYP2E1 have been identified (http://www.imm.ki.se/CYPalleles/cyp2e1.htm). Among these alleles, the most common ones that are associated with high inter individual variation and those that influence gene function and expression have been investigated in this study. These genes (*CYP2E1*1B*, *CYP2E1*6* and *CYP2E1*5B*) have been extensively analyzed for their polymorphic distribution in other major ethnic groups but till date there are no documentations for the same in our population.

CYP2E1*1B or TaqI polymorphism in intron 7 has a

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base change at 9896C>G. The polymorphism results in enhanced activity of CYP2E1 enzyme in vivo (Haufroid et al., 2001). CYP2E1*6 or DraI polymorphism in intron 6 with a base change at 7632T>A is associated with reduced CYP2E1 activity (Haufroid et al., 2002). CYP2E1*5B constitutes RsaI (-1053C>T) and PstI (-1293G>C) polymorphisms in the 5'promoter region of the gene affects the transcriptional regulation of the gene (Hayashi et al., 1991). These two RFLPs are in complete linkage disequilibrium and associated with reduced activity of the enzyme (Watanabe et al., 1990; Haufroid et al., 2002).

Glutathione S-transferases are a family of phase II detoxification enzymes. These enzymes play a central role in the detoxification of many endogenous and exogenous substrates through conjugation to glutathione, a tripeptide consisting of glycine, glutamic acid, cysteine to electrophilic compounds, resulting in less reactive and more easily excretable glutathione conjugates. Among the 3 mammalian GSTs, (mitochondrial, cytosolic and microsomal) cytosolic GSTs represent the largest family and exhibits significant genetic polymorphism. The different isoenzymes of cytosolic GSTs are Mu, Theta, Pi, Sigma, Omega, Alpha and Zeta. It has been reported that deficient genotypes or polymorphism in GST Mu (M1), Theta (T1), and Pi (P1) contributes to increased susceptibility to various diseases (Townsend and Tew 2003; Hayes et al., 2005). Among the isoenzymes, GSTP1 is also of particular interest because it is widely expressed in a variety of tumors. The efficacy and toxicity of anticancer agents differs greatly among patients based on their GSTP1 genotypes as the enzyme is involved in the metabolism of certain chemotherapeutics.

The null genotypes of GSTM1 and T1 in Tamilian population have already been established by our co-workers (Naveen et al., 2004). However, data on GSTP1 genotype has not yet been recorded in our population. *GSTP1 is* located on chromosome 11q13. The genotype *(Ile/Val)* constitutes an A313G change, causing an Ile to Val substitution at amino acid 105 within the active site of the enzyme which results in substantial reduction of enzyme activity by altering its catalytic activity (Townsend and Tew 2003).

Tamilnadu, one among the four South Indian states has about 6.14% of Indian population as on 2001 (http://www.statoids.com/uin.html). Tamilians, belonging to the Dravidian race, were the first major occupants of the country and settled in the north-western part of India. This ethnic group is markedly different from north Indians who are Aryan descendents. Both these groups have different cultural, linguistic and dietary practices.

Hence, the purpose of our study was to establish the prevalence of *CYP2E1*1B, CYP2E1*6, CYP2E1*5B* and *GSTP1 (Ile/Val)* genotypes in Tamilnadu population. Since the frequency of these genetic polymorphisms expected to vary between various ethnic groups, the data will be useful for further epidemiological studies.

Materials and Methods

The subjects chosen for the study were healthy, unrelated volunteers belonging to a single race, whose parental origin was from different regional parts of Tamilnadu. Written informed consent was obtained from all the subjects. The study was approved by Institutional Ethics Committee.

Subjects analyzed for *CYP2E1* gene include 123 (95 males and 28 females), with a mean age of (±SD) 33.65 ± 12.02 years. The TaqI polymorphism (A2A2 wild type) (Hu et al., 1997), DraI polymorphism (DD wild type) (Mattias et al., 1998) and two polymorphisms containing RsaI and PstI sites (c1c1 wild type) (Mattias et al., 1998) were identified by PCR-RFLP methods with a few modifications with respect to PCR conditions.

The subjects for *GSTP1* polymorphism (Ile/Ile wild type) included 133 (109 males and 24 females), had a mean age

Table 1. Genotyping Details of CYP2E1 & GSTP1

Gene	Primers	Conditions	Enzymes	Size of PCR product (bp)
CYP2E1*1B	F- 5' GGA TGA TGG GTG GAT GCC 3' R -5' CAC ATG TGG AGG GGA GAT 3'	94ºC-2min 94ºC-30sec, 58ºC -1min, 72ºC -1 min x 30 cycles	TaqI	A2A2-639 + 330 A2A1-969+639+330 A1A1-969
CYP2E1*5B	F- 5' CCA GTC GAG TCT ACA TTG TCA 3' R -5' TTC ATT CTG TCT TCT AAC TGG 3'	94ºC-2min 94ºC-20sec, 55ºC -30sec, 72ºC -30sec x 30 cycles	RsaI/PstI	RsaI c1c1-352+61 c1c2-413+352+61 c2c2-413 PstI c1c1-413 c1c2-413+118+295 c2c2-118+295
CYP2E1*6	F-5' AGT CGA CAT GTG ATG GAT CCA 3' R-5' GAC AGG GTT TCA TCA TGT TGG 3'	94ºC-2min 94ºC-20sec, 64ºC -30sec, 72ºC -30sec x 30 cycles	DraI	DD-251+125 DC-376+251+125 CC-376
GSTP1	F-5' ACC CCA GGG CTC TAT GGG AA 3' R-5' TGA GGG CAC AAG AAG CCC CT 3'	94ºC-2min 94ºC-20sec, 59ºC -30sec, 72ºC -30sec x 30 cycles	BsmAI	Ile/Ile-176 Ile/Val-176+83+93 Val/Val—83+93

of (±SD) 33.5±11.85 years. The polymorphism was detected using PCR-RFLP method (Kote et al., 2001). Table 1 summarizes the primers, PCR conditions and restriction enzymes used for the genotype analysis of CYP2E1 and GSTP1.

5 ml of blood was collected using ethylene diamine tetraacetic acid (EDTA) as anticoagulant. Genomic DNA was extracted from the peripheral leucocytes using standard phenol: chloroform method. The PCR mixture (50µL) was prepared containing 30–50ng of DNA, 5µL of 10X buffer (500mM KCl / 100mM tris-HCl pH 8.3/ 15 Mm MgCl2), 1µL of 2.5 mM dNTPs, 0.5µL of 20 pmol each of the primers, 2 U of Taq polymerase (Supratherm). PCR product of 10 µL was digested using appropriate restriction enzymes. The digested PCR products were separated by electrophoresis using 8% polyacrylamide gel and stained with ethidium bromide.

Genotyping procedures were validated by sequencing of representative samples by the dideoxymediated chain-termination method of Sanger et al using the ABI Prism-377 sequencer and ABI Prism BigDye-Terminator v3.0 cycle sequencing.

Statistical analysis was performed using the Graphpad Instat statistical software (GraphPad Software Inc., San Diego, CA, USA). The data was analyzed by Fisher's exact test and $P < 0.05$ was considered statistically significant. The observed genotype frequencies were compared with the expected frequencies to check for the Hardy–Weinberg equilibrium.

Results

The genotype distribution of CYP2E1*1B, CYP2E1*6, CYP2E1*5B in Tamilian population are given in Table 2. The frequency distribution of *CYP2E1*1B* A2A2, A2A1 and A1A1 genotypes were 61%, 36% and 3% respectively. The distribution of *CYP2E1*5B* c1c1, c1c2 genotypes were 99.2% and 0.8%. C*YP2E1*6* DD, DC and CC genotype frequencies were 72%, 25% and 3% respectively. The allele frequencies of CYP2E1*1B, CYP2E1*5B and CYP2E1*6 were A2- 0.79 A1- 0.21, c1-0.996 c2 - 0.004 and D- 0.84 C-0.16 respectively.

Table 3 summarizes the genotype frequency data of *GSTP*1 *(Ile/Val)* polymorphism. The genotype frequency of *GSTP1* was Ile/Ile-44%, Ile/Val-47%, Val/Val-9% whereas; the allelic frequencies were 0.67 for Ile and 0.33 for Val allele. Figure 1 shows the representative gel pictures of the genotypes, CYP2E1*1B, CYP2E1*6, CYP2E1*5B and GSTP1 Ile/Val respectively. The genotype distributions of both the genes were consistent with Hardy-Weinberg equilibrium.

Discussion

The present study is the first report on the genotype distribution of *CYP2E1*1B* in the Asian population. A significant decrease in percentage distribution of genotype frequencies were observed with *A2A2* and *A2A1* of *CYP2E1*1B* in this population in comparison with

Table 2. Genotype Distribution of CYP2E1 in Tamilnadu Population Compared to Other Populations

CYP2E1 genotypes	Tamilians		Caucasians		Taiwanese		North Indians	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
CYP2E1*1B	123		375[†]					
A2A2	75	61 (52.4-69.6)	279	75** (70-78.8)				
A2A1	44	35.8 (27.3-44.2)	91	24* (4.1-9.3)	NR		NR	
A1A1	4	3.2 (0.8-8.1)	5	1 (0.4-3.1)				
		279						
CYP2E1*5B	123		1454[‡]		320[§]		227[††]	
c1c1	122	99.2 (95.6-100)	1344	92.4*** (90.9-93.7)	198	61.9*** (56.6-67.2)	223	98.2 (95.6-99.5)
c1c2	1	0.80 (0.02-4.5)	109	7.5*** (6.2-8.9)	113	35.3*** (30.1-40.5)	4	1.8 (0.48-4.4)
c2c2	0	0 (0-3)	1	0.1 (0.006-0.4)	9	2.8 (1.3-5.3)	0	0 (0-1.6)
CYP2E1*6	123		1360[‡]		320[§]		227[††]	
DD	88	71.5 (63.6-79.5)	1162	85.4*** (83.6-87.3)	183	57.2** (51.8-62.6)	147	64.8 (58.5-71)
DC	31	25.25 (17.5-32.9)	187	13.8** (11.9-15.6)	123	38.4** (33.1-43.8)	73	32.2 (26.1-38.2)
CC	4	3.25 (0.8-8.1)	11	0 .8* (0.4-1.4)	14	4.4 (2.4-7.2)	7	3.08 (1.3-6.3)

n= No. of subjects, NR-Not reported ***P<0.001; **P<0.01; *P<0.05 when compared to Tamilians

([†]- Wong et al., 2000; [‡]- Garte et al., 2001; [§]- Hildesheim et al., 1997; [††] - Mittal et al., 2005)

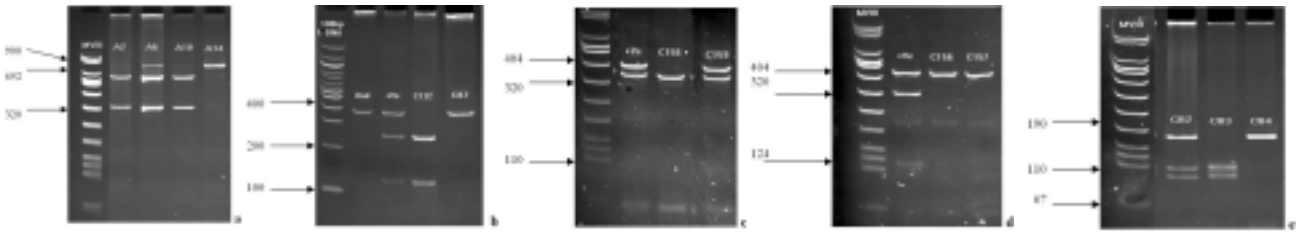

Figure 1. Representative Gel Pictures of CYP2E1*1B (Fig 1a), CYP2E1*6 (Fig 1b), CYP2E1*5B (Fig 1c & d) and GSTP1 (Fig 1e) assays. (1a) CYP2E1*1B assay: Lane 1 is molecular weight marker VIII, lanes 2 & 4 - A2A2, lane 3 - A2A1 and lane 5 - A1A1. (1b) CYP2E1*6 assay: Lane 1 is 100 bp DNA ladder, lane 2- PCR product that has not been digested by DraI enzyme, lane 3- DC, lane 4- DD and lane 5 - CC genotype. (1c-RsaI) CYP2E1*5B assay: Lane 1 is molecular weight marker VIII, lane 2 &4 - c1c2 and lane 3 is c1c1. (1d-PstI) CYP2E1*5B assay: Lane 1 is molecular weight marker VIII, lane 2 - c1c2 and lanes 3 & 4 represent c1c1. (1e) GSTP1 (Ile/Val) assay: Lane 1 is molecular weight marker VIII, lane 2- Ile/Val, lane 3- Val/Val and lane 4 represents Ile/Ile genotype.

Caucasians (Wong *et al*., 2000) (Table 2). The frequency of *CYP2E1*5B c1c1* and *CYP2E1 *6* genotypes lies between Caucasians and Taiwanese (Hildesheim et al., 1997; Garte et al., 2001). However, the distribution of rare alleles of these polymorphisms did not differ significantly (Table 2).

Documentation of this data on the frequency distribution of *CYP2E1* genotypes gains importance as the enzyme is of major toxicological interest. It metabolizes several precarcinogens, drugs and solvents to reactive metabolites which ultimately lead to DNA or protein damage (Carriere et al., 1995). Hence, the data on the prevalence of these polymorphisms will help in predicting susceptibility to various cancers and liver diseases.

Observation from the present study on the genotype distribution of *GSTP1* (Ile/Ile) and (Ile/Val) reveals that it varies significantly from Chinese Orientals ($p<0.05$) (Tan et al., 2000) but not different from Caucasians (Kote et al., 2001) (Table 3). This was in agreement with the data analyzed in north Indian population where they found a significant difference from Japanese who are an integral part of Orientals (Mishra et al., 2004).

GSTP1 is a major enzyme metabolizing anti cancer drugs like oxiplatin, cyclophosphamide which are used in the treatment of breast cancer and colorectal cancer (Marsh and McLeod 2004). An over expression of this enzyme in individuals with Ile/Ile genotype causes resistance to drugs like cisplatin in oral and maxillofacial squamous carcinoma (Xu et al., 2002; Townsend and Tew 2003). Therefore, investigation of these polymorphisms will provide a clue to the identification of responders to cancer therapy with certain chemotherapeutic drugs.

We also found a similar distribution in genotypes of *CYP2E1* and *GSTP1* polymorphisms compared with North Indian population (Mishra et al., 2004; Mittal et al., 2005) even though these populations are ethnically diverse. The CYP2E1 findings were in contrary to the earlier reports from our laboratory which had established that the frequencies of other CYP enzymes like *CYP2C19* and *CYP2D6* genes in Tamilian population differed significantly from North Indians (Adithan et al., 2003a, b). However, the distribution of *GSTP1* in both the Indian populations were in agreement with the earlier reports from north India, (Mishra et al., 2004) where they observed a similar distribution of *GSTM1* and *T1* null alleles compared to south Indians which was already been established by our co-workers (Naveen et al., 2004).

To conclude, this study provides the first results of genotype distribution of *CYP2E1* and *GSTP1* in South Indian population. The study opens up new avenues for further investigations by epidemiologists in determining inter-individual variation in genetic susceptibility to various diseases caused due to gene- environment interaction.

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Table 3. Genotype Distribution of GSTP1 in Tamilnadu Population in Comparison with Other Populations

GSTP1 genotypes	Tamilians		Caucasians		Chinese		North Indians	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
	133		273†		150‡		370§	
Ile/Ile	58	43.6 (35.2-52)	140	51.3 (45.4-57.2)	91	60.7** (52.8-68.5)	164	44.3 (39.3-49.4)
Ile/Val	63	47.4 (38.9-5.9)	105	38.5 (32.7-44.2)	53	35.3* (27.7-43)	186	50.3 (45.2-55.4)
Val/Val	12	9.0 (4.75-15.2)	28	10.3 (6.66-13.9)	6	4.0 (1.48-8.5)	20	5.4 (3.34-8.2)

**P<0.01; *P<0.05 when compared to Tamilians n= No. of subjects († - Kote et al., 2001; ‡ - Tan et al., 2000; §- Mishra et al., 2004)

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