RESEARCH ARTICLE

Functional PstI/RsaI Polymorphisms in the *CYP2E1* Gene among South Indian Populations

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Abstract

Human cytochrome P4502E1 (CYP2E1) is a well-conserved xenobiotic-metabolizing enzyme expressed in liver, kidney, nasal mucosa, brain, lung, and other tissues. CYP2E1 is inducible by ethanol, acetone, and other low-molecular weight substrates and may mediate development of chemically-mediated cancers. *CYP2E1* polymorphisms alter the transcriptional activity of the gene. This study was conducted in order to investigate the allele frequency variation in different populations of Andhra Pradesh. Two hundred and twelve subjects belonging to six populations were studied. Genotype and allele frequency were assessed through TaqMan allelic discrimination (rs6413419) and polymerase chain reaction-sequencing (-1295G>C and -1055C>T) after DNA isolation from peripheral leukocytes. The data were compared with other available world populations. The SNP rs6413419 is monomorphic in the present study, -1295G>C and -1055C>T are less polymorphic and followed Hardy-Weinberg equilibrium in all the populations studied. The -1295G>C and -1055C>T frequencies were similar and acted as surrogates in all the populations. Analysis of HapMap populations data revealed no significant LD between these markers in all the populations. Low frequency of *CYP2E1c2 could be useful in the understanding of south Indian population gene composition, alcohol metabolism, and alcoholic liver disease development. However, screening of additional populations and further association studies are necessary. The heterogeneity of Indian population as evidenced by the different distribution of *CYP2E1**c2 may help in understanding the population genetic and evolutionary aspects of this gene.**

Keywords: *CYP2E1* - SNP - allelic variation - South Indian populations

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Introduction

Drug metabolizing enzymes are involved in the processing of xenobiotics to expel them easily from the body and occasionally they also mediate the toxicity or carcinogenicity through the metabolic activation of procarcinogens. CYP2E1 is a well-conserved xenobiotic-metabolizing cytochrome P450 (CYP) enzyme. *CYP2E1* is expressed in liver, kidney, nasal mucosa, brain, lung, and other tissues (Sohda et al., 1993). CYP2E1 expression is easily inducible by a variety of physiological and pathophysiological conditions, ethanol, acetone, and other low-molecular weight substrates. Induction of CYP2E1 is the first step involved in the development of chemically-mediated cancers. CYP2E1 metabolically activates a large number of low relative molecular mass toxicants and carcinogens and thus is of enormous toxicological and carcinogenic importance (Gonzalez, 2007). Since the bioactivation of carcinogens through CYP is the first step of crucial importance to the cancer development, a large number of studies have been carried out to investigate the association between the *CYP2E1* polymorphisms and risk of various cancers, but findings have been inconsistent (Klinchid et al., 2009; Hata et al., 2010; Wang et al., 2010; Ye et al., 2010; Balaji et al., 2011; Sameer et al., 2011). Inhibition of CYP2E1 using tolfenamic acid attenuated the bioactivation of carcinogens potentially leading to the chemoprevention (Shukoor et al., 2012) and hence the development of effective CYP2E1 inhibitors, proved to be effective in preventing CYP2E1-mediated cancers.

The *CYP2E1* gene is mapped to chromosome 10q24.3-qter. The gene spans over 11 kb and contains 9 exons coding for a membrane-bound protein consisting of 493 amino acid residues with a molecular weight of ~ 57 kDa (Lewis et al., 1997). Both the 5'-flanking region (5'-FR) and 3'-untranslated-region (3'-*UTR*) harbour several mutations known to alter the transcriptional activity of the gene (Hayashi et al., 1991; Chen et al., 2006). Two

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point mutations in the 5'-FR PstI and RsaI- which are in close linkage disequilibrium are known to generate the *CYP2E1*_1 (c1) allele and the less common *CYP2E1*_2 (c2) allele. PstI and RsaI have been associated with a greater risk for oral, pharyngeal (Matthias et al., 2002; Gattas et al., 2006), liver (Hata et al., 2010; Ye et al., 2010) and lung cancers (Klinchid et al., 2009; Wang et al., 2010). It is well established that frequencies of *CYP2E1* polymorphisms differ markedly in among different ethnic and racial groups. Attempts have been made to study the *CYP2E1* polymorphisms at global level (Lee et al., 2008) and the Indian population diversity provides immense opportunity in this regard. Therefore, in this study, the frequency of three important *CYP2E1* polymorphisms p. V179I (dbSNP rs6413419), -1295G>C (dbSNP rs3813867) and -1055C>T (dbSNP rs2031920) in 5'-flanking region and T7678A polymorphism in intron 6, were investigated in six south Indian populations.

Materials and Methods

Populations

The study population included a total of 212 males belonging to six ethnic groups of south India. All the subjects were apparently normal healthy volunteers and no diagnosis of alcoholism or related disorders performed on them. About 3 ml of blood samples were collected with the informed written consent of all the participants. The study was approved by the Ethics Committee of Narayana Medical College, Nellore, India. DNA of the above samples was isolated using the standard protocol (Sambrook et al., 1989).

Genotyping

The SNP rs6413419 genotyping was performed using a pre-designed TaqMan allelic discrimination assay (part number: C__30443971_10, Applied Biosystems, Foster City, CA, USA). The PCR was carried out in a final reaction volume of 5 µL which contained 2.5 µL TaqMan Universal PCR Master Mix, 0.125 µL TaqMan SNP Genotyping Assay, 1.375 µL distilled water and 1 µL DNA (10 ng/µL). After PCR, the genotype of each sample was automatically attributed by measuring the allele-specific fluorescence in the ABI 7900HT Sequence Detection System, using the SDS 2.3 software for allele discrimination (Applied Biosystems, Foster City, CA, USA). The single nucleotide polymorphisms -1295G>C and -1055C>T in 5'-flanking region was genotyped using PCR sequencing. The forward 5'-ccagtcgagtctacattgtca-3' and reverse 5'-ttcattctgtcttctaactgg-3' primers were used to amply 413bp product. PCRs were carried out in a Verity thermocycler (Applied Biosystems) in 0.2 ml tubes using 10 ml standard reaction volume. PCR amplicons were checked on 2% agarose gel prior to DNA sequencing.

Statistical analysis

Allele frequencies at each locus in all populations were determined by direct gene counting. The genotype distribution for each site in each sample was evaluated for departure from Hardy-Weinberg equilibrium using the HWSIM program (Cubells et al., 1997). Within each population, haplotype frequencies were obtained from three-site and six-site haplotypes by summing frequencies of each relevant haplotype. Haplotypes were estimated from phase-unknown multi-site genotypes based on a maximum likelihood method employing an EM algorithm using Arlequin 2.0 (Schneider et al., 2000). Linkage disequilibrium (D' and r^2) was estimated using HaploView 3.12 (Barrett et al., 2005). To understand the global distribution of *CYP2E1* -1295G>C and -1055C>T, we have also extracted 20kb up and downstream SNPs around these SNPs from HapMap data (Tanaka, 2009).

Results

Out of three SNPs analysed only two were polymorphic in the study populations. All 212 samples analysed for rs6413419 showed GG genotype, indicating that this SNP is monomorphic in the study population. Hence we excluded this SNP from further analysis. The genotypes of -1295G>C and -1055C>T SNPs in 5' -flanking region are designated as c1/c1 for wild type homozygous, c1/c2 for heterozygous and c2/c2 for mutant homozygous individuals. Both -1295G>C and -1055C>T SNPs remained highly polymorphic in the studied populations. Genotype frequencies of both polymorphisms fit Hardy-Weinberg equation. Population-specific genotype counts and frequencies among different populations were shown in Table 1. Although there is no much difference in the minor allele among populations both, SNPs co-inherited in the populations. The -1295G>C and -1055C>T SNPs, which are in complete linkage disequilibrium, was found to be polymorphic in four out of six populations. The homozygous wild type genotype (c1/c1) frequency was 88.2% in Madiga to 97.8% in Reddy populations whereas the heterozygous genotype (c1/c2) was 2.3% in Muslim to 11.8% in Madiga population. None of the population showed homozygous mutants genotypes (c2c2). The minor allele frequency of -1295G>C and -1055C>T SNPs varied from 1.1% in Reddy to 5.9% in Madiga populations. Haplotype analysis using -1295G>C and -1055C>T SNPs were provided in Table 2. The pairwise LD values (D'=1 and r^2=1) between rs3813867 and rs2031920 also revealed that one SNP can act as a

Table 1. Genotype, Allele Frequencies for SNPs rs3813867 and rs2031920 among 6 Indian Populations

Population	c1/c1	c1/c2	c2/c2	c1 allele	c2 allele	HWE p value
Reddy	45 (97.83)	1 (2.17)	0 (0)	0.989	0.011	0.941
Sugali	27 (100)	0 (0)	0 (0)	0	0	NA
Balija	33 (97.06)	1 (2.94)	0 (0)	0.985	0.015	0.931
Muslim	42 (97.67)	1 (2.33)	0 (0)	0.988	0.012	0.939
Mala	45 (100)	0 (0)	0 (0)	1	0	NA
Madiga	15 (88.24)	2 (11.76)	0 (0)	0.941	0.059	0.797

Table 2. CYP2E1 Gene Haplotypes in Different Populations

	rs3813867 and rs2031920					
Haplotype	Reddy	Sugali	Balija	Muslim	Mala	Madiga
CT	0.011	0	0.015	0.012	0	0.059
GC	0.989	1	0.985	0.988	1	0.941

surrogate for another. The minor allele frequencies of rs6413419 and c2 allele in different HapMap populations were documented in Table 3. Calculation of LD in the 20kb up and downstream regions from -1055C>T (dbSNP rs2031920) loci in HapMap populations revealed that the east Asian populations showed larger LD blocks followed by the European populations (Figure S1). These blocks are very small in African populations.

Discussion

In this study, rs6413419, -1295G>C and -1055C>T polymorphisms are analyzed for 212 individuals belong to 6 Indian populations. The rs6413419 is not polymorphic in study populations. The -1295G>C and -1055C>T are identified in four populations and are completely in strong linkage disequilibrium with each other. A homozygous mutated individual was not identified in this study. East Asian populations showed larger LD blocks compared to the other populations.

Table 3. Minor Allele Frequencies of CYP2E1 Gene rs2031920 and rs6413419 in Different HapMap Populations

Label	Population	c2 allele	rs6413419
ASW	African ancestry in Southwest USA	0.024	0.223
CEU	Utah residents with European ancestry from the CEPH collection	0.103	0.021
CHB	Han Chinese in Beijing, China	0.289	0
CHD	Chinese in Metropolitan Denver, Colorado	0.306	0
GIH	Gujarati Indians in Houston, Texas	0.023	0.011
JPT	Japanese in Tokyo, Japan	0.291	0
LWK	Luhya in Webuye, Kenya	0	0.15
MEX	Mexican ancestry in Los Angeles, California	0.247	0.032
MKK	Maasai in Kinyawa, Kenya	0	0.088
TSI	Toscans in Italy	0.102	0.04
YRI	Yoruba in Ibadan, Nigeria	0	0.316

*MAF; Minor allele frequency

CYP2E1 is one of the xenobiotic-metabolizing P450s and is involved in the interindividual variations in susceptibility to occupational diseases caused by chemicals or chemical-induced carcinogenesis. Like most other xenobiotic-metabolizing genes, *CYP2E1* polymorphisms frequency differs distinctly among ethnic and racial groups (Evans and Relling, 1999). Although, the molecular basis of the human *CYP2E1* regulation largely remains unknown, previous studies has shown that the c2 mutant allele has been related to an increase in *CYP2EI* expression. The DNA containing *CYP2E1* gene c2 mutation showed 10 times better expression as compared to the expression in native DNA in HepG2 cells (Hayashi et al., 1991). Furthermore, Individuals with c2 allele showed 1.7-fold higher *CYP2E1* mRNA content than the c1 allele carriers indicating that RsaI polymorphism affects the binding of a transcription factor and the transcriptional activation of *CYP2E1* (Watanabe et al., 1994).

The c2 mutant allele frequency in study populations is 1.1-5.9% and is consistent with previous reports from India (Sikdar et al., 2003; Krishnakumar et al., 2012). The c2 mutant allele in South Indians was lower than in Chinese, Taiwanese, Japanese and Caucasians populations (Garte et al., 2001). The c2 mutant allele frequency varies in the European populations ranges from 3.47% to 5.5% (Tang et al., 2010). Analysis of Brazilian populations revealed variations in the c2 mutant allele frequency between white and non-white individuals (Rossini et al., 2006). The c2 allele frequency is higher in Mexicans as compared with other Native American populations (Mendoza-Cantu et al., 2004). The c2 allele frequency in Turkish population is almost similar to the Caucasian populations (Omer et al., 2001; Ulusoy et al., 2007). Analysis of three Ethnic groups from Russia revealed significant differences between the populations with the c2 allele ranged from 2.66%

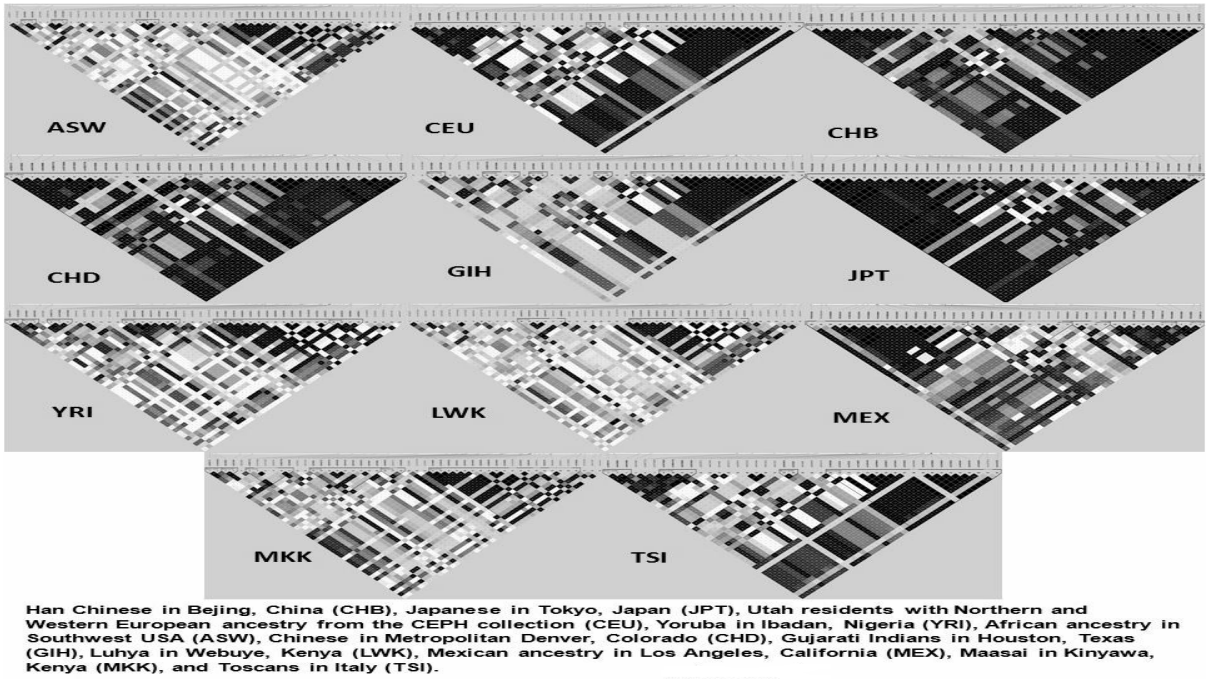

Figure 1. Linkage Disequilibrium Profiles in Different World Populations Studied in International HapMap Project. Colour coding represents the D'/LOD values and the values in cells are r^2 multiplied by 100

in Russians to 6.8% in Bashkirs (Korytina et al., 2012). Analysis of HapMap data revealed that the frequency of c2 mutant allele varies from population to population. Caucasians representatives of HapMap, Utah residents with Northern and Western European ancestry from the CEPH collection (CEU) and Toscans in Italy showed the frequency of 10.3% and 10.2% respectively. The HapMap Chinese populations, Chinese Han Chinese in Beijing (CHB) and Chinese in Metropolitan Denver (CHD) showed the frequency of 28.9% and 30.6% respectively. The c2 allele in Japanese (JPT) is -29.1% and in Mexicans (MEX) 24.7%. The African populations representing the HapMap (LWK, MKK and YRI) did not show the c2 allele.

In view of the significant inter-ethnic variations in *CYP2E1* polymorphisms, it was of great interest to establish their genotype and allele frequency spectrum in Indian populations. Subsequently, this information could be useful in assessing the genetic risk factors to cancer susceptibility and other diseases associated with detoxification pathway. Thus, it is recommended that a larger population is screened to confirm the results found in the present study and to relate these data to the geographical differences in diseases associated with *CYP2E1* polymorphisms in India.

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