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Prevalence of *CYP2C8* polymorphisms in a North Indian population

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ABSTRACT. CYP2C8 is an important member of the cytochrome P450 family of enzymes; it affects the activity of various drugs used in routine clinical practice, including amiodarone, chloroquine, amodiaquine, and repaglinide, as well as endogenous compounds, such as arachidonic acid and retonic acid. It is also the main enzyme involved in the metabolism of the widely used anticancer drug Paclitaxel, which has a very narrow therapeutic index. There is evidence that single nucleotide polymorphisms in the *CYP2C8* gene influence the adverse reactions and/or the efficacy of drugs metabolized by this enzyme. We examined the allele and genotype frequencies of widely studied functional polymorphisms of the *CYP2C8* gene in a North Indian population. We assayed the genomic DNA of at least 251 healthy unrelated North Indians for *CYP2C8*2*, *CYP2C8*3* (G416A, A1196G), and *CYP2C8*4* genetic polymorphisms by RFLP technique. These results were compared to information on other populations. The allelic frequencies of *CYP2C8*2*, *CYP2C8*3*, and *CYP2C8*4* were found to be 3, 4, and 4% respectively. The two *CYP2C8*3* polymorphisms (G416A and A1196G) were found to be completely linked to each other. Allele frequencies of *CYP2C8* genetic variants in northern Indians were found to have a distinct pattern that differs from that of southern

Indian and other global populations. This is the first report from North India on *CYP2C8* polymorphisms. Ethnic differences with respect to polymorphisms are the molecular basis of interethnic variability in pharmacokinetics. Our study may help in rational use of drugs that are substrates for CYP2C8 in this population.

Key words: Pharmacogenetics; Single nucleotide polymorphisms; Cytochrome P450; *CYP2C8*

INTRODUCTION

Cytochrome P450 (CYP) is composed of a large and very important group of enzymes responsible for the metabolism of xenobiotics and endogenous compounds. It is a superfamily of proteins that originated approximately 3 billion years ago and is found in almost every class of organism (Danielson, 2002). The cytochrome P450 2C (CYP2C) subfamily of enzymes constitutes approximately 18-30% of all human CYPs (Goldstein, 2001). CYP2C8 is an important member of the CYP2C subfamily of enzymes, as it is responsible for metabolism of various drugs used in routine clinical practice.

CYP2C8, along with other members of its subfamily, notably CYP2C9, CYP2C18, and CYP2C19, metabolize approximately 20% of clinically prescribed drugs (Gray et al., 1995; Evans and Relling, 2004). Of all the members, however, CYP2C8 is the major enzyme responsible for metabolism of several clinically relevant drugs, including paclitaxel, amodiaquine, chloroquine, repaglinide, and amiodarone, as well as many others. A full list of substrates metabolized by CYP2C8 is shown in Table 1. Several polymorphisms have been identified within the gene encoding CYP2C8, which is located on the long arm of chromosome 10. Genetic polymorphisms are thought to be the molecular basis responsible for the ethnic variability in drug response and adverse reactions. Previous studies have suggested that polymorphisms in *CYP2C8* could result in both positive and negative clinical consequences, in terms of altered pharmacokinetics of drugs that are metabolized by CYP2C8.

Table 1. Substrates metabolized by CYP2C8.

Role of CYP2C8	Broad category of drugs	Specific drugs
Major role	Oncology and hematology	Paclitaxel, all-*trans*-retinoic acid
	Antimalarials	Amodiaquine, chloroquine
	Cardiovascular system	Amiodarone, cerivastatin
	Antidiabetics	Repaglinide, rosiglitazone, pioglitazone, troglitazone
	Others	Tazarotenic acid (acne and psoriasis)
Intermediate or minor role	Oncology and hematology	Cyclophosphamide, ifosfamide
	Cardiovascular system	Diltiazem, fluvastatin, simvastatin acid, verapamil
	Anti-inflammatory agents/analgesics	Diclofenac, ibuprofen, methadone, morphine, tenoxicam
	Others	Carbamazepine (antiepileptic), dapsone (leprosy and pneumocystis pneumonia), loperamide (antidiarrheal), torsemide (edema), zopiclone (insomnia)

*Adapted from previously published articles by Daily and Aquilante (2009) and Totah and Rettie (2005).

Apart from the wild-type allele *CYP2C8*1*, three other variant alleles, designated as *CYP2C8*2* (A805T), *CYP2C8*3* (G416A, A1196G), and *CYP2C8*4* (C792G) are present

among several ethnic populations. These three variants exist in the coding regions of *CYP2C8* and are the most frequently studied polymorphisms of the gene. The *CYP2C8*2* variant allele results in an Ile269Phe substitution in exon 5 and is the most common variant of *CYP2C8* in Africans. The two polymorphisms of *CYP2C8*3*, G416A, and A1196G, result in an Arg139Lys substitution in exon 3 and a Lys399Arg substitution in exon 8, repectively. Both *CYP2C8*2* and *CYP2C8*3* were associated with the defective metabolism of the widely used anticancer drug paclitaxel *in vitro* (Dai et al., 2001). The resultant polymorphic enzyme generated from the CYP2C8*4 Ile264Met substitution in exon 5 has only 25% activity relative to the wild-type *CYP2C8* enzyme (Jiang et al., 2011).

The spectrum of single nucleotide polymorphisms in *CYP2C8* differs among ethnic populations. The frequency of the various polymorphisms of this gene has been studied in a number of populations, including South Indians, Caucasians, Chinese, Japanese, and Africans, to name a few. To our knowledge, there has been no report to date of any *CYP2C8* polymorphisms in North Indians. The North Indian population largely consists of Caucasoids that speak Indo-European languages (Indian Genome Variation Consortium, 2005). The aim of the present study was to determine the allele and genotype frequencies of *CYP2C8*2*, *CYP2C8*3*, and *CYP2C8*4* genetic polymorphisms in a North Indian population.

MATERIAL AND METHODS

Subjects

A total of 254 healthy and unrelated North Indian human subjects residing in Delhi and its adjoining cities for the past three generations were recruited for upon visiting Sir Ganga Ram Hospital, in New Delhi, India, for a routine health check-up. All subjects gave written informed consent. A brief medical history of every subject was taken to ensure good subject health. The study was approved by the Ethics Committee of the Sir Ganga Ram Hospital.

Genotyping

Four milliliters of venous blood was obtained from each subject, from which DNA was subsequently isolated using a QIAamp DNA Blood Mini Kit (QIAGEN, Germany).

Genotyping of all four polymorphisms of *CYP2C8* was done using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)-based assays, with Taq polymerase (Genetix Biotech, India) and a GeneAmp PCR System 9700 (Applied Biosystems, USA). *CYP2C8*2* and *CYP2C8*3* (A1196G) genotypes were analyzed, with minor modifications, in methods previously described by Dai et al. (2001), while *CYP2C8*3* (G416A) and *CYP2C8*4* genotypes were analyzed, with minor modifications, in methods previously described by Nakajima et al. (2003). PCR conditions, primer sets, and the restriction enzymes used are summarized in Table 2.

Statistical analysis

Expected allelic frequencies were calculated with the Hardy-Weinberg equilibrium (HWE) test using the HWE software (Rodriguez et al., 2009) and, subsequently, the χ^2 test was

used to compare the observed and expected genotype frequencies. The distribution of allele frequencies in our study population was compared with that of previously reported populations, also with the χ^2 test. Values with $P < 0.05$ were considered to be significant.

Table 2. PCR conditions, primer sets and restriction enzymes.

CYP2C8 allele	Nucleotide change	Resulting amino acid change	Annealing temperature (°C)	Restriction enzyme	Fragment length (bp)	Electrophoresis
*CYP2C8*2*	805 A→T	Ile269Phe	54	*Bcl*I	wt: 214, 98 mut: 312	2% agarose
*CYP2C8*3* (G416A)	416 G→A	Arg139Lys	59	*Bse*RI	wt: 310, 157 mut: 467	2% agarose
*CYP2C8*3* (A1196G)	1196 A→G	Lys399Arg	55	*Xmn*I	wt: 92, 25 mut: 117	12% polyacrylamide
*CYP2C8*4*	792 C→G	Ile264Met	50	*Taq*I	wt: 83, 53, 31 mut: 136, 31	12% polyacrylamide

Primer sequences
*CYP2C8*2*: forward: 5'-AAGATACATATATCTTATGACATG-3'; reverse: 5'-ATCCTTAGTAAATTACAGAAGG-3'
*CYP2C8*3* (G416A): forward: 5'-AGGCAATTCCCCAATATCTC-3'; reverse: 5'-CAGGATGCGCAATGAAGAC-3'
*CYP2C8*3* (A1196G): forward: 5'-CTTCCGTGCTACATGATGACG-3'; reverse: 5'-CTGCTGAGAAAGGCATGAAG-3'
*CYP2C8*4*: forward: 5'-AAAGTAAAAGAACACCAAGC-3'; reverse: 5'-AAACATCCTTAGTAAATTACA-3'

wt = wild-type allele; mut = mutant-type allele.

RESULTS

Genotyping of *CYP2C8* genetic polymorphisms was carried out in at least 251 North Indian subjects. The frequencies of *CYP2C8*2*, *CYP2C8*3*, and *CYP2C8*4* variant alleles in this population were 3, 4, and 4%, respectively. The allele frequencies were in HWE for all of the *CYP2C8* polymorphisms tested. The detailed distribution of genotypes is shown in Table 3.

Table 3. Distribution of *CYP2C8* genotypes in the North Indian population.

CYP2C8 allele	N	Observed genotypes	Expected values of genotypes by HWE	P value
*CYP2C8*2*	251	A/A: 237	A/A: 237.2	0.65
	A/T: 14	A/T: 13.61		
	T/T: 0	T/T: 0.2		
*CYP2C8*3* (G416A)	254	G/G: 235	G/G: 234.39	0.31
	G/A: 18	G/A: 19.21		
	A/A: 1	A/A: 0.39		
*CYP2C8*3* (A1196G)	254	A/A: 235	A/A: 234.39	0.31
	A/G: 18	A/G: 19.21		
	G/G: 1	G/G: 0.39		
*CYP2C8*4*	251	C/C: 233	C/C: 233.32	0.55
	C/G: 18	C/G: 17.35		
	G/G: 0	G/G: 0.32		

No homozygous variant carriers were found with respect to *CYP2C8*2* and *CYP2C8*4*. Only one subject was a carrier of a homozygous variant *CYP2C8*3* allele. *CYP2C8*3* polymorphisms G416A and A1196G were found to be linked together in all the subjects. The comparison of allele frequencies between this North Indian population and previously reported populations is summarized in Table 4.

Table 4. Allele frequencies of *CYP2C8* in North Indians and comparison with other major previously reported populations.

CYP2C8 allele	Population	No. of subjects	Allele frequency (%)
*CYP2C8*2*	North Indians (Present study)	251	3.0
	South Indians (Arun et al., 2011)	245	0.8*
	Malaysian Indians (Muthiah et al., 2005)	123	0.8
	Caucasians (British) (Bahadur et al., 2002)	116	0.4*
	Africans (Ghanaian) (Kudzi et al., 2009)	203	17.0#
	Japanese (Nakajima et al., 2003)	360	0.0#
*CYP2C8*3*	North Indians (Present study)	254	4.0
	South Indians (Arun et al., 2011)	245	2.9
	Malaysian Indians (Muthiah et al., 2005)	123	1.2*
	Caucasians (British) (Bahadur et al., 2002)	107	15.0#
	Africans (Ghanaian) (Kudzi et al., 2009)	204	0.0#
	Japanese (Nakajima et al., 2003)	360	0.0#
*CYP2C8*4*	North Indians (Present study)	251	4.0
	Malaysian Indians (Muthiah et al., 2005)	123	0.0*
	Caucasians (British) (Bahadur et al., 2002)	107	7.5
	Africans (Ghanaian) (Kudzi et al., 2009)	204	0.0#
	Japanese (Nakajima et al., 2003)	360	0.0#

*$P < 0.05$ compared with the North Indians. #$P < 0.001$ compared with the North Indians.

DISCUSSION

The present study investigated the frequency of the three most common variant alleles of the *CYP2C8* gene in a North Indian population. All three variants, *CYP2C8*2*, *CYP2C8*3*, and *CYP2C8*4*, were found in our population.

The *CYP2C8*2* variant allele results in the defective metabolism of CYP2C8 substrates, which in turn could lead to an increased drug half-life and subsequent related adverse effects (Totah and Rettie, 2005). Indeed, Parikh et al. (2007) previously demonstrated that this allele leads to the defective metabolism of the antimalarial drug Amodiaquine. Furthermore, this variant has recently been shown to be associated with *P. falciparum* chloroquine-resistant infections (Paganotti et al., 2011). The *CYP2C8*2* variant allele was found at a frequency of 3% in our study population. The prevalence of this allele in the North Indian population is significantly higher than in the previously reported South Indian population ($P < 0.05$). North Indians, therefore, may be more susceptible than South Indians to the adverse effects this allele causes with respect to drugs metabolized by the CYP2C8 enzyme. The observed difference in the prevalence of this polymorphism between North and South Indians may be related to the underlying ethnic differences between the origins of two populations. North Indians are considered to be Caucasoids while South Indians are considered to be Australoids (Indian Genome Variation Consortium, 2005). The incidence of the *CYP2C8*2* variant allele in North Indians was also considerably higher than it is in the British Caucasian population ($P < 0.05$) and Asians ($P < 0.001$), but the prevalence was significantly lower than that found in Africans ($P < 0.001$).

The presence of the *CYP2C8*3* allele has been shown to impact the clearance of paclitaxel in ovarian cancer patients (Gréen et al., 2009; Bergmann et al., 2011). Subsequently, it is associated with paclitaxel-induced neurotoxicity (Leskelä et al., 2011) and a complete response from neoadjuvant paclitaxel treatment in breast cancer patients (Hertz et al., 2012). *CYP2C8*3* has also been shown to play an important role in drug-drug interactions (Gao et al., 2010). Most recently, it has been shown to influence the gemfibrozil-pioglitazone drug interaction (Aquilante et al., 2012). In the present study, the *CYP2C8*3* variant allele was found at a

frequency of 4% in a North Indian population. This frequency is significantly lower than that of Caucasians ($P < 0.001$), while the *CYP2C8*3* variant allele is not found at all in African and Japanese populations. The two polymorphisms of this allele, G416A and A1196G, were found to be completely linked together, in agreement with findings from previously published studies on other populations (Dai et al., 2001; Pechandova et al., 2012).

The *CYP2C8*4* variant allele is not present among Malaysian Indian, African, and Japanese populations (Nakajima et al., 2003; Muthiah et al., 2005; Kudzi et al., 2009). In the present study, its frequency was 4% in a North Indian population. Bahadur et al. (2002) identified the *CYP2C8*4* variant allele at a frequency of 7.5% in Caucasians. *CYP2C8*4* is associated with a lower incidence of lymph node involvement in breast cancer patients (Jernström et al., 2009) and was also shown to influence paclitaxel pharmacokinetics in a study of ovarian cancer patients (Bergmann et al., 2011). Importantly, not all studies have found an association between the paclitaxel response and/or toxicity and *CYP2C8* genotypes (Marsh et al., 2007). According to one report, *CYP2C8*4* along with *CYP2C8*3* can impact the metabolism of diclofenac *in vivo* (Dorado et al., 2008).

In conclusion, we have estimated the prevalence of *CYP2C8* polymorphisms in a healthy North Indian population. These findings may lead to a better understanding of pharmacogenetics and the appropriate dosing of drugs that are metabolized by the CYP2C8 enzyme, in the population studied. To our knowledge, this is the first study to document the distribution of *CYP2C8* polymorphisms in a North Indian population.

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