

# Occurrence of CYP2B6 516G>T polymorphism in patients with ARV-associated hepatotoxicity

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## Funding information

The study was supported by a research grant provided by NARI-ICMR India.

## Abstract

**Background:** Hepatic enzyme cytochrome P450 2B6 (CYP2B6) plays a role in the metabolism of efavirenz drugs. *CYP2B6* 516G>T variation showed an implication for HIV treatment.

**Methods:** *CYP2B6* 516G>T polymorphism was genotyped in a total 165 HIV patients that include 34 with and 131 without hepatotoxicity and 155 healthy individuals by the PCR-RFLP.

**Results:** In patients with hepatotoxicity, the prevalence of *CYP2B6* 516TT genotype was higher as compared to healthy individuals (35.3% vs. 30.5%, OR = 1.74). Patients with hepatotoxicity using tobacco had a higher prevalence of genotypes *CYP2B6* 516GT, 516TT, 516GT+TT as compared to healthy individuals (28.57% vs. 25.93%; 57.14% vs. 29.63%; 85.71% vs. 55.56%). Likewise, hepatotoxicity in patients consuming alcohol showed higher distributions of *CYP2B6* 516GT, 516TT, 516GT+TT genotypes (57% vs. 25.93%; 42.86% vs. 33.33%; 71.43% vs. 59.26%). Nevirapine users with hepatotoxicity overrepresented genotypes *CYP2B6* TT and 516GT+TT as compared to efavirenz users (47.83% vs. 45.45%, OR = 6.88, 65.22% vs. 54.55%, OR = 1.56). Similarly, in nevirapine +alcohol users with hepatotoxicity, the frequency of *CYP2B6* 516GT, 516GT+TT genotypes was higher than with nevirapine +alcohol nonusers (40.0% vs. 11.11%, OR = 8.00, 80.0% vs. 27.78%, OR = 4.00). In HIV patients, nevirapine users had higher frequency of *CYP2B6* 516GT, 516GT+TT genotypes as compared to efavirenz users (42.02% vs. 25.00%, OR = 2.53; 72.27% vs. 58.33%, OR = 1.86). Likewise, in HIV patients, genotypes *CYP2B6* 516GT, 516GT+TT were predominant with nevirapine +alcohol users as compared to nevirapine +alcohol nonusers (57.89% vs. 34.57%, OR = 2.46; 78.95% vs. 69.14%, OR = 1.67). In multivariate logistic regression, taking nevirapine had a protection for severity of ARV-associated hepatotoxicity (OR = 0.23,  $p = 0.005$ ).

**Conclusions:** No significant association was detected between *CYP2B6* 516G>T polymorphism and susceptibility to ARV-associated hepatotoxicity.

## KEYWORDS

ARV-associated hepatotoxicity, CYP2B6, genetic polymorphism, HIV patients, NNRTI drugs

## 1 | INTRODUCTION

Antiretroviral therapy (ART) has modified the natural history of HIV infection in both rich and limited-resource countries (LRCs) like India. Two lines of treatment are widely available to treat the HIV patients and almost no test, aside from the CD4 cell count, is performed for monitoring treatment outcome. Long-term efficacy and toxicity are major challenges when selecting an antiretroviral (ARV) regimen for treatment of AIDS. A common adverse drug reaction (ADR) is hepatotoxicity which leads to treatment interruptions in HIV patients (Dyke, Wang, & Williams, 2008). Individuals using nevirapine were associated with higher incidence of liver toxicity than efavirenz (van Leth et al., 2004). A 10.8% grade 3 or 4 hepatotoxicity was observed with efavirenz-treated group and 8.9% in the nevirapine-treated group (Reisler, Servoss, & Sherman, 2001). However, the incidence of hepatotoxicity due to nevirapine was 3.19% (Nagpal, Tayal, Kumar, & Gupta, 2010).

Mainly, drug metabolism and detoxification enzymes can be present in the liver and these are susceptible to injury. Single nucleotide polymorphism (SNP) in drug metabolizing enzyme genes may cause liver toxicity (Bissell, Gores, Laskin, & Hoofnagle, 2001). CYP2B6 (OMIM: 614,546) is an isoenzyme found on exon 4 of chromosome 19 and is characterized by wide interindividual and interethnic variation in expression and activity in the human liver in vitro (Saitoh et al., 2007). Drug metabolizing enzyme including CYP2B6 plays a major role in the hydroxylation of EFV and NVP (Erickson, Mather, Trager, Levy, & Keirns, 1999; Ward et al., 2003). A total of 29 alleles of CYP2B6 (\*1A [wild-type] to \*29) have been described (CYP2B6 allele nomenclature. [www.cypalleles.ki.se/cyp2b6.htm](http://www.cypalleles.ki.se/cyp2b6.htm) 102), many of which are associated with increased, decreased, or abolished enzyme activity (Watanabe et al., 2010; Zanger et al., 2007; Zhang et al., 2011). CYP2B6\*6 516G>T and 785A>G polymorphism were associated with “slow metabolism” of EFV (Zhang et al., (2011); Desta et al., 2007). The CYP2B6\*6 516G>T gene polymorphism is accountable for aberrant splicing, resulting in a high-splice variant and low CYP2B6 expression phenotype (Hofmann et al., 2008). The CYP2B6 516 G>T gene polymorphism is associated with higher plasma EFV concentrations leading to increased drug-related side effects (Haas et al., 2005; Nyakutira et al., 2008; Ribaldo et al., 2006; Rodriguez-Novoa et al., 2005; Sadiq, Fredericks, Khoo, Rice, & Holt, 2005). A G>T change at position 516 of CYP2B6 gene results in a Gln–His (Glutamine–Histidine) amino acid change which is associated with a significant reduction in CYP2B6 catalytic activity (Penzak et al., 2007). The 516TT genotype of CYP2B6 is associated with reduced enzyme activity (Gatanaga et al., 2007).

Studies have reported an association between CYP2B6 genotypes and the pharmacokinetics of EFV and NVP in

HIV-infected patients of European and African ethnicities (Cabrera et al., 2009; Haas et al., 2009; Mahungu et al., 2009; Motsinger et al., 2006; Ribaldo et al., 2007; Rotger et al., 2007; Schipani et al., 2011; Wyen et al., 2008). Also, similar studies have been published in other ethnically diverse patients (Carr, Porte, Pirmohamed, Owen, & Cortes, 2010; Chen et al., 2010; Lindfelt, O'Brien, Song, Patel, & Winslow, 2010; Puthanakit, Tanpaiboon, Aurlpibul, Cressey, & Sirisanthana, 2009; Ramachandran et al., 2009; To et al., 2009). In India, it is believed that we have different gene pools in south, north, east, and west regions. The frequency of CYP2B6 516G>T polymorphism was reported only in South Indian population (Ramachandran et al., 2009). Hence, we evaluated the prevalence of CYP2B6 516G>T polymorphism in West Indian HIV patients.

## 2 | MATERIAL AND METHODS

### 2.1 | Subjects

This is a case–control study, undertaken between November 2012 and February 2015, at the outpatient clinics of National AIDS Research Institute, Pune. The study included 34 patients with hepatotoxicity (Grade III/IV) under NNRTI containing ART regimen, 131 HIV patients without hepatotoxicity confirmed by liver function test (LFT), and 155 age-matched healthy controls. Patients with hepatotoxicity having hepatitis B, hepatitis C, tuberculosis, and concurrent untreated opportunistic infections, immune reconstitution syndrome and under any other known hepatotoxic drugs were excluded from cases. HIV patients having evidence of hepatotoxicity, hepatitis B, hepatitis C, tuberculosis, and receiving any other known hepatotoxic drugs were excluded. One hundred and fifty-five individuals (those from the same family were excluded), HIV, Hepatitis B, C and Tuberculosis free, age-matched and serum negative from HIV-ELISA test were recruited. Clinical research proforma was filled to obtain clinical data by questionnaire, personal interviews, and review of case records. Liver function test was done to evaluate the status of liver enzyme. Total Bilirubin >3.22 mg/ml, SGOT >93.8 U/ml, SGPT >229.5 U/ml, and Alkaline phosphatase >550.8 U/ml for male hepatotoxicity cases and total Bilirubin >3.22 mg/ml, SGOT >163.2 U/ml, SGPT >173.4 U/ml, and Alkaline phosphatase >550.8 U/ml for female hepatotoxicity were considered as cases. Total Bilirubin <1.24 mg/ml, SGOT <32 U/ml, SGPT <34 U/ml, and Alkaline phosphatase <108 U/ml for male and female HIV-infected control were considered. Estimation of CD4 count was done by fluorescence-activated cell sorter (FACS). CD4 status was used to classify patients into different subgroups. CD4 ranges from <200 cells/mm<sup>3</sup> were defined as an advanced stage, 201–350 cells/mm<sup>3</sup> as an intermediate stage, and >350 cells/mm<sup>3</sup> onward as an early stage. ELISA for

hepatitis C and HBsAg testing was performed using Ortho HCV ELISA test system and Murex HBsAg confirmatory (Diasorin) ELISA. Environmental exposures such as tobacco and alcohol usage were also recorded in the questionnaire. The study was approved by the local institutional ethics committee and written informed consent was taken from all eligible participants.

### 3 | DNA EXTRACTION

Two milliliters of peripheral blood sample was collected and stored at  $-70^{\circ}\text{C}$  prior to DNA extraction. Genomic DNA extraction was done from peripheral blood leukocytes pellet using the AxyPrep Blood Genomic DNA Miniprep Kit according to the protocol given by the manufacturer.

#### 3.1 | Genotyping

The *CYP2B6* (516G>T) was genotyped using PCR-restriction fragment length polymorphism (PCR-RFLP) in subjects participants. The amplification of *CYP2B6* (516G>T) primers was taken as reported (Ramachandran et al., 2009). PCR was performed in a total volume of 20  $\mu\text{l}$  with 20 pmol of each primer, genomic DNA (100–150 ng), 10 mmol/L deoxynucleotide triphosphates, PCR buffer containing 100 mmol/L Tris-HCl, pH 8.6, 50 mmol/L KCl, 1.5 mmol/L  $\text{MgCl}_2$ , and 1.5 units of Taq polymerase (Bangalore Genei, India). The reaction conditions for *CYP2B6* 516G>T were: initial denaturation at  $94^{\circ}\text{C}$  for 3 min, followed by 35 cycles of denaturation at  $95^{\circ}\text{C}$  for 30 s, annealing at  $58^{\circ}\text{C}$  for 30 s, extension at  $72^{\circ}\text{C}$  for 45 s, and final extension at  $72^{\circ}\text{C}$  for 7 min. Amplified product of *CYP2B6* was digested using restriction enzyme *BsrI* (Fermentas Inc.). Genotyping of *CYP2B6* was done in 15% polyacrylamide gel using molecular weight markers and visualized after staining with ethidium bromide. Based on sequences and location of SNP, genotypes of *CYP2B6* were as assigned as follows: for *CYP2B6*: 204 bp for 516GG (good metabolizer); 152 and 52 bp for 516TT (poor metabolizer), and 204, 152, and 52 bp for 516GT (intermediate metabolizer) genotype. Veriti 96-well Thermal cycler (Applied Biosystems) was used to amplify the desired DNA. PCR products and molecular weight markers were visualized after staining with ethidium bromide. Twenty percent of samples from both patients and controls were re-genotyped by other laboratory personnel and no discrepancy in genotyping was noticed. Ten percent of samples were sequenced to assess the genotyping error.

#### 3.2 | Data analysis

The age variable was expressed as mean  $\pm$  SD. The deviation from Hardy–Weinberg equilibrium in controls was

analyzed using  $\chi^2$  goodness-of-fit test. We compared the genotype frequency between HIV patients with hepatotoxicity versus without hepatotoxicity and HIV patients versus healthy controls using  $\chi^2$  statistic (Fisher's exact test for cell size  $<5$ ). Tobacco, alcohol usages, and genotypes interaction were examined in all eligible HIV patients. Odds ratios (ORs) and 95% confidence interval (CI) were calculated by unconditional binary logistic regression. All statistical analysis was performed using SPSS software version 17.0 (SPSS) and tests of statistical significance were two-sided and taken as significant when  $p$ -value was less than 0.05.

### 4 | RESULTS

The study consisted of a total 165 HIV patients of which 34 patients had hepatotoxicity, 131 were without hepatotoxicity, and 155 were healthy controls. The mean age (years  $\pm$ SD) of patients with hepatotoxicity, without hepatotoxicity, and healthy controls were 39.45 years  $\pm$  3.91, 38.39 years  $\pm$  4.23, and 36 years  $\pm$  2.80, respectively. The demographic profile of HIV patients with, without hepatotoxicity, and healthy controls is shown in Table 1.

### 5 | GENOTYPE–PHENOTYPE CORRELATION

#### 5.1 | CYP2B6 516G/T polymorphism and HIV patients with hepatotoxicity

The genotype and allele frequency of *CYP2B6* 516G>T polymorphism in total HIV patients and healthy controls and HIV patients with and without hepatotoxicity are shown in Table 2. The distribution of *CYP2B6* 516G>T polymorphism was not significantly different between total HIV patients and healthy controls. Higher distribution of *CYP2B6* 516TT genotype was observed in HIV patients with hepatotoxicity as compared to without hepatotoxicity (35.3% vs. 30.5%, OR = 1.74, 95%CI: 0.66–4.60,  $p = 0.25$ ). The *CYP2B6* 516GT genotype and 516T allele were not significantly different between both the groups (26.5% vs. 40.5% and 48.5% vs. 50.8%, respectively).

#### 5.2 | CYP2B6 516G/T polymorphism and HIV patients

The distribution of *CYP2B6* 516G>T polymorphism in HIV patients and healthy controls is presented in Table 3. *CYP2B6* 516G>T polymorphisms in healthy controls followed the Hardy–Weinberg equilibrium ( $p = 0.12$ ). The *CYP2B6* carriage 516TT genotype presented slightly higher in HIV patients as compared to healthy controls (30.5% vs. 27.1%, OR = 1.14, 95%CI: 0.63–2.06,  $p = 0.64$ ). The difference in frequency of *CYP2B6* 516GT genotype and 516T allele was

**TABLE 1** Characteristics of HIV patients with hepatotoxicity, without hepatotoxicity, and healthy controls

S. no.	HIV patients with hepatotoxicity (Grade III and IV)	HIV patients without hepatotoxicity	Healthy controls
Number	<i>N</i> = 34	<i>N</i> = 131	<i>N</i> = 155
Mean age (range)	40.70 ± 7.63	39.41 ± 7.18	36.75 ± 8.50
Females	15 (44.11%)	47 (35.87%)	38 (25%)
Males	19 (55.88%)	84 (64.12%)	117 (75.48%)
NNRTI regimen			
Nevirapine <i>N</i> = 142	30 (88.23%)	112 (85.49%)	Not applicable (NA)
Efavirenz <i>N</i> = 23	4 (11.76%)	19 (14.50%)	NA
Alcohol habit			
User <i>N</i> = 51	23 (67.64%)	28 (21.37%)	13 (8.12%)
Nonuser <i>N</i> = 114	11 (32.35%)	103 (78.62%)	21 (13.12%)
Tobacco habit			
User <i>N</i> = 50	23 (67.64%)	27 (20.61%)	15 (9.37%)
Nonuser <i>N</i> = 115	11 (32.35%)	104 (79.38%)	27 (16.87%)
CD4+ status			
<200 ( <i>N</i> = 95)	25 (73.52%)	61 (45.56%)	NA
201–350 ( <i>N</i> = 50)	9 (26.47%)	50 (38.16%)	NA
>350 ( <i>N</i> = 20)	0 (0)	20 (15.26%)	NA

**TABLE 2** Frequency distribution of *CYP2B6* 516G/T genotypes in a total HIV patients and healthy controls and HIV patients with and without hepatotoxicity

Genotypes <i>CYP2B6</i> 516 G/T	Total HIV patients <i>N</i> = 165 (%)	Healthy controls <i>N</i> = 155 (%)	<i>p</i> -value	OR (95%CI)
GG	51 (30.90)	45 (29.0)	1	Reference
GT	62 (37.57)	68 (43.9)	0.50	0.80 (0.46–1.41)
TT	52 (31.51)	42 (27.1)	0.87	1.09 (0.59–2.01)
GT+TT	114 (69.09)	110 (71.0)	0.89	1.00 (0.58–1.73)
Alleles	Total HIV patients <i>N</i> = 330 (%)	Healthy controls <i>N</i> = 310 (%)	<i>p</i> -value	OR (95%CI)
G	164 (49.69)	158 (51.0)	1	Reference
T	166 (50.30)	152 (49.0)	0.80	1.05 (0.76–1.45)
Genotypes <i>CYP2B6</i> 516 G/T	HIV patients with hepatotoxicity <i>N</i> = 34 (%)	HIV patients without hepatotoxicity <i>N</i> = 131 (%)	<i>p</i> -value	OR (95%CI)
GG	13 (38.2)	38 (29.0)	1	Reference
GT	9 (26.5)	53 (40.5)	0.67	0.82 (0.32–2.07)
TT	12 (35.3)	40 (30.5)	0.25	1.74 (0.66–4.60)
GT+TT	21 (61.8)	93 (71)	0.40	0.66 (0.28–1.56)
Alleles	HIV patients with hepatotoxicity <i>N</i> = 68 (%)	HIV patients without hepatotoxicity <i>N</i> = 262 (%)	<i>p</i> -value	OR (95%CI)
G	35 (51.5)	129 (49.2)	1	Reference
T	33 (48.5)	133 (50.8)	0.78	1.04 (0.80–1.35)

Note. *N* = number of subjects, (%) = frequency of genotypes/allele. Presence of GG for GT, TT, and GT+TT genotypes and G for T allele were taken as reference group for statistical analysis.

<sup>a</sup>Chromosome 19 - NC\_000019.10.

**TABLE 3** Frequency distribution of *CYP2B6* 516 G/T genotypes in HIV patients and healthy controls

Genotypes <i>CYP2B6</i> 516 G/T	HIV patients	Healthy controls	<i>p</i> -value	OR (95%CI)
	<i>N</i> = 131(%)	<i>N</i> = 155 (%)		
GG	38 (29.0%)	45 (29.0%)	1	Reference
GT	53 (40.5%)	68 (43.9%)	0.98	0.99 (0.52–1.87)
TT	40 (30.5%)	42 (27.1%)	0.64	1.14 (0.63–2.06)
GT+TT	93(71.0%)	110 (71.0%)	0.89	1.00 (0.58–1.73)
Alleles	HIV patients	Healthy controls	<i>p</i> -value	OR(95%CI)
	<i>N</i> = 262(%)	<i>N</i> = 310 (%)		
G	129 (49.2%)	158 (51.0%)	1	Reference
T	133 (50.8%)	152 (49.0%)	0.68	0.93 (0.67–1.29)

Note. *N* = number of subjects, (%) = frequency of genotypes/allele. Presence of GG for GT, TT genotypes and G for T allele were taken as reference group for statistical analysis.

**TABLE 4** Frequency distribution of *CYP2B6* 516G/T genotypes in different HIV disease stages and healthy controls

Genotypes <i>CYP2B6</i> 516 G/T	Healthy controls <i>N</i> = 155 (%)	Early HIV disease stage		Intermediate HIV disease stage		Advanced HIV disease stage	
		<i>N</i> = 19 (%)	OR (P)	<i>N</i> = 33 (%)	OR (P)	<i>N</i> = 79 (%)	OR (P)
GG	45 (29.0%)	7 (36.84%)	1 (Reference)	11 (33.34%)	1 (Reference)	20 (25.32%)	1 (Reference)
GT	68 (43.9%)	4 (21.05%)	0.38 (0.22)	14 (42.42%)	0.84 (0.87)	35 (44.30%)	1.16 (0.79)
TT	42 (27.1%)	8 (42.11%)	1.22 (0.93)	8 (24.24%)	0.78 (0.81)	24 (30.38%)	1.29 (0.62)

Note. *N* = number of subjects, (%) = frequency of genotypes. Presence of GG for GT, TT genotypes were taken as reference group for statistical analysis.

not statically significant between HIV patients and healthy controls (40.5% vs. 43.9% and 50.8% vs. 49.0%, respectively).

### 5.3 | Genotype-HIV disease stage correlation

*CYP2B6* 516G>T polymorphism was not significantly distributed between HIV disease stages and healthy controls. However, the presence of *CYP2B6*516TT genotype was greater in advanced HIV disease stages compared with healthy controls (30.38% vs. 27.1%, OR = 1.29, 95%CI: 0.58–2.84, *p* = 0.62) (Table 4).

### 5.4 | Gene–environment interaction

In patients with hepatotoxicity along with tobacco usage genotypes *CYP2B6*516GT, 516TT and 516GT+TT genotypes were higher as compared to nonusers (28.57% vs. 25.93%, OR = 3.43, 95%CI: 0.18–117.72, *p* = 0.73; 57.14% vs. 29.63%, OR = 6.00, 95%CI: 0.45–170.81, *p* = 0.27; 85.71% vs. 55.56%, OR = 4.80, 95%CI: 0.44–121.06, *p* = 0.30, respectively). Similarly, the frequency of distribution of genotype *CYP2B6*516GT was higher in HIV patients using tobacco as compared to nonusers (46.51% vs. 37.50%, OR = 1.31, 95%CI: 0.50–3.48, *p* = 0.70) (Table-5).

The occurrence of *CYP2B6*516GT, 516TT, and 516GT+TT genotypes was higher in patients with hepatotoxicity using

alcohol than in nonusers (28.57% vs. 25.93%, OR = 1.57, 95%CI: 0.12–21.71, *p* = 0.68; 42.86% vs. 33.33%, OR = 1.83, 95%CI: 0.18–20.78, *p* = 0.92; 71.43% vs. 59.26%, OR = 1.72, 95%CI: 0.22–15.77, *p* = 0.87, respectively). Likewise, the occurrence of *CYP2B6*516GT and 516GT+TT genotypes was higher in HIV patients with alcohol users as compared to nonusers (54.54% vs. 33.33%, OR = 2.03, 95%CI: 0.77–5.43, *p* = 0.17; 74.99% vs. 68.97%, OR = 1.35, 95%CI: 0.55–3.33, *p* = 0.60, respectively) (Table 5)

Table 6 shows the distribution of *CYP2B6* 516G>T polymorphism in patients with hepatotoxicity, without hepatotoxicity using nevirapine and efavirenz. In patients with hepatotoxicity, the distribution of *CYP2B6*516TT, 516GT+TT genotypes appeared to be higher in nevirapine users as compared to efavirenz users (47.83% vs. 9.09%, OR = 6.88, 95%CI: 0.55–189.71, *p* = 0.19; 65.22% vs. 54.55%, OR = 1.56, 95%CI: 0.29–8.64, *p* = 0.82, respectively). Similarly, *CYP2B6*516GT, 516TT, 516GT+TT genotypes were observed predominantly at a higher level in HIV patients with nevirapine use as compared to efavirenz use (42.02% vs. 25.00%, OR = 2.53, 95%CI: 0.48–14.49, *p* = 0.38; 30.25% vs. 33.33%, OR = 1.36, 95%CI: 0.28–6.74, *p* = 0.93; 72.27% vs. 58.33%, OR = 1.86, 95%CI: 0.47–7.17, *p* = 0.49, respectively).

In patients with hepatotoxicity *CYP2B6*516GT, 516TT, 516GT+TT genotypes were found to be much prevalent in nevirapine +alcohol users as compared to nevirapine +alcohol



nonusers (40.0% vs. 11.11%, OR = 8.00, 95%CI: 0.28–449.98,  $p = 0.41$ ; 40.0% vs. 44.44%, OR = 2.00, 95%CI: 0.10–69.78,  $p = 0.92$ ; 80.0% vs. 27.78%, OR = 4.00, 95%CI: 0.31–110.70,  $p = 0.46$ , respectively). In HIV patients, *CYP2B6* 516GT, 516GT+TT genotypes were distributed at a higher level with nevirapine +alcohol users in comparison to nevirapine +alcohol nonusers (57.89% vs. 34.57%, OR = 2.46, 95%CI: 0.84–7.31,  $p = 0.10$ ; 78.95% vs. 69.14%, OR = 1.67, 95%CI: 0.62–4.62,  $p = 0.37$ , respectively) (Table 7).

## 5.5 | Risk factors of ARV-associated hepatotoxicity: Multivariate logistic regression analysis

Correlation of age, sex, tobacco, alcohol, baseline CD4 counts, and *CYP2B6* 516G/T polymorphism with ARV-associated hepatotoxicity were done by multivariate logistic

regression analysis. *CYP2B6* 516G/T polymorphism, age, sex, tobacco, alcohol usage, and baseline CD4 counts were not independent risk factors for ARV-associated hepatotoxicity. While comparing between HIV patients with and without hepatotoxicity, *CYP2B6* 516TT genotype, taking alcohol and male HIV patients with hepatotoxicity showed a risk for ARV-associated hepatotoxicity (OR = 1.79,  $p = 0.27$ ; OR = 1.91,  $p = 0.63$ ; OR = 1.90,  $p = 0.16$ , respectively) (Table 8).

## 6 | DISCUSSION

The differences in the activity of drug metabolizing enzyme contribute to interpatient variability in response to drugs. The SNPs of CYP450 enzymes are known to have a role in influencing the efficacy of therapy (Servoss et al., 2006). The

**TABLE 5** Frequency distribution of *CYP2B6* 516 G/T genotypes in HIV patients with and without hepatotoxicity using tobacco and alcohol

Genotypes <i>CYP2B6</i> 516 G/T	Tobacco user	Tobacco nonuser	<i>p</i> -value	OR 95% CI
	<i>N</i> = 7 (%)	<i>N</i> = 27 (%)		
HIV patients with hepatotoxicity				
GG	1 (14.29%)	12 (44.44%)	1	Reference
GT	2 (28.57%)	7 (25.93%)	0.73	3.43 (0.18–117.72)
TT	4 (57.14%)	8 (29.63%)	0.27	6.00 (0.45–170.81)
GT+TT	6 (85.71%)	15 (55.56%)	0.30	4.80 (0.44–121.06)
Genotypes <i>CYP2B6</i> 516 G/T	Tobacco user	Tobacco nonuser	<i>p</i> -value	OR 95% CI
	<i>N</i> = 43 (%)	<i>N</i> = 88 (%)		
HIV patients without hepatotoxicity				
GG	12 (27.91%)	26 (29.55%)	1	Reference
GT	20 (46.51%)	33 (37.50%)	0.70	1.31 (0.50–3.48)
TT	11 (25.58%)	29 (32.95%)	0.88	0.82 (0.28–2.43)
GT+TT	31 (72.09%)	62 (70.45%)	0.99	1.08 (0.45–2.63)
Genotypes <i>CYP2B6</i> 516 G/T	Alcohol user	Alcohol nonuser	<i>p</i> -value	OR 95% CI
	<i>N</i> = 7 (%)	<i>N</i> = 27 (%)		
HIV patients with hepatotoxicity				
GG	2 (28.57%)	11 (40.74%)	1	Reference
GT	2 (28.57%)	7 (25.93%)	0.68	1.57 (0.12–21.71)
TT	3 (42.86%)	9 (33.33%)	0.92	1.83 (0.18–20.78)
GT+TT	5 (71.43%)	16 (59.26%)	0.87	1.72 (0.22–15.77)
Genotypes <i>CYP2B6</i> 516 G/T	Alcohol user	Alcohol nonuser	<i>p</i> -value	OR 95% CI
	<i>N</i> = 44 (%)	<i>N</i> = 87 (%)		
HIV patients without hepatotoxicity				
GG	11 (25.00%)	27 (31.03%)	1	Reference
GT	24 (54.54%)	29 (33.33%)	0.17	2.03 (0.77–5.43)
TT	9 (20.45%)	31 (35.63%)	0.69	0.71 (0.23–2.22)
GT+TT	33 (74.99%)	60 (68.97%)	0.60	1.35 (0.55–3.33)

Note. *N* = number of subjects, (%) = frequency of genotypes. Presence of GG for GT, TT genotypes were taken as reference group for statistical analysis.

**TABLE 6** Frequency distribution of *CYP2B6* 516 G/T genotypes in NNRTI regimens using HIV patients with and without hepatotoxicity

Genotypes <i>CYP2B6</i> 516 G/T	Nevirapine user N = 23 (%)	Efavirenz user N = 11 (%)	p-value	OR 95% CI
HIV patients with hepatotoxicity				
GG	8 (34.78%)	5 (45.45%)	1	Reference
GT	4 (17.39%)	5 (45.45%)	0.72	0.50 (0.06–3.82)
TT	11 (47.83%)	1 (9.09%)	0.19	6.88 (0.55–189.71)
GT+TT	15 (65.22%)	6 (54.55%)	0.82	1.56 (0.29–8.64)
Genotypes <i>CYP2B6</i> 516 G/T	Nevirapine user N = 119 (%)	Efavirenz user N = 12 (%)	p-value	OR 95% CI
HIV patients without hepatotoxicity				
GG	33 (27.73%)	5 (41.67%)	1	Reference
GT	50 (42.02%)	3 (25.00%)	0.38	2.53 (0.48–14.49)
TT	36 (30.25%)	4 (33.33%)	0.93	1.36 (0.28–6.74)
GT+TT	86 (72.27%)	7 (58.33%)	0.49	1.86 (0.47–7.17)

Note. N = number of subjects, (%) = frequency of genotypes. Presence of GG for GT, TT and GT+TT genotypes were taken as reference group for statistical analysis.

SNPs within the *CYP2B6* gene are associated with altered hepatic *CYP2B6* expression and activity (Tong et al., 2006). The interindividual differences in hepatic *CYP2B6* expression and enzymatic activity may result in variable systemic exposure and therapeutic response to the drugs metabolized by *CYP2B6*.

The *CYP2B6* gene is highly polymorphic (Lang et al., 2001) and encodes the major enzymes for hepatic elimination of efavirenz and nevirapine. The prevalence of T allele varies within the populations (Tong et al., 2006; Xu et al., 2007). The variant 516 T allelic is more common in African-Americans (20%) than in Hispanics (6.7%), or Caucasians (3.4%) and has been reproducibly associated with slower clearance and ethnic difference in efavirenz plasma concentrations (Haas et al., 2009). Ethnicity plays a key role, as reflected by the variation in frequency of T allele across the populations. In this study, the distribution of *CYP2B6* 516 G>T polymorphism was not significantly different among HIV patients and healthy controls, patients with hepatotoxicity versus without hepatotoxicity. In our study in the West Indian, a prevalence of 30.5% was observed for 516TT genotype in healthy controls as compared to 42% represented in South India (Servoss et al., 2006). The distribution of T allele frequency was 16% in the Japanese (Hiratsuka et al., 2002), 14% in Koreans (Cho et al., 2004), 42% in West Africans, 62% in Papua New Guineans (Mehlotra et al., 2011), 28% in white Americans, 25% Caucasians (Haas et al., 2009), 28% African-Americans (Haas et al., 2009), 43% and 34.5% Chinese (Tong et al., 2006; Xu et al., 2007), 43% Hispanics (Haas et al., 2009), 42% South India (Servoss et al., 2006), and 49.0% in this study.

In this is a case–control study, the current CD4 count was considered as a substitute of current HIV disease stage. As

the time points for HIV infection were not known, the results may be confounded by the duration of HIV infection. In our study, the distribution of *CYP2B6* 516G/T polymorphism was not significantly different between HIV disease stages and healthy controls.

The gene–environment interactions determine the pathophysiology of the disease (Deng, Newman, Dunne, Silburn, & Mellick, 2004). However, for case–control association studies for environmental influences, cases must have matched controls (Greenland, 1980). It is assumed that a case study is always better to look at for the effect of gene–environment. Here, we have chosen the case-only analysis. Heavy alcohol consumption had a negative impact on the CD4 cell counts of HIV patients naïve to ART (Samet et al., 2007). In our study, *CYP2B6*516TT genotype modulated the severity risk in patients with hepatotoxicity using tobacco (OR = 6.00,  $p = 0.27$ ). Hepatotoxicity risk was revealed among alcohol users, with *CYP2B6*516TT genotype (OR = 1.83,  $p = 0.92$ ). Similarly, presence of *CYP2B6* 516GT genotype among alcohol users resulted in the modulation of risk for acquisition of ARV-associated hepatotoxicity (OR = 2.03,  $p = 0.17$ ).

*CYP2B6* 516TT genotype also indicated a risk for hepatotoxicity severity in nevirapine users (OR=6.88,  $p = 0.19$ ). Likewise, *CYP2B6* 516TT genotype among combined nevirapine +alcohol users has shown a risk for hepatotoxicity severity (OR = 2.00,  $p = 0.92$ ). *CYP2B6* 516GT+TT combined genotype revealed a risk for acquisition of ARV-associated hepatotoxicity and its severity in combined alcohol and nevirapine users (OR = 1.67,  $p = 0.37$ ; OR = 4.00,  $p = 0.46$ , respectively). In multivariate logistic regression, while comparing between HIV patients with and without hepatotoxicity, *CYP2B6* 516TT genotype showed a risk for ARV-associated hepatotoxicity (OR = 1.79,  $p = 0.27$ ).It supports the idea that

**TABLE 7** Frequency distribution of *CYP2B6* 516 G/T genotypes in combined NNRTI regimen using HIV patients with and without hepatotoxicity

Genotypes <i>CYP2B6</i> 516 G/T	Nevirapine + alcohol user N = 5 (%)	Nevirapine + alcohol nonuser N = 18 (%)	p-value	OR 95% CI
HIV patients with hepatotoxicity				
GG	1 (20.0%)	8 (44.44%)	1	Reference
GT	2 (40.0%)	2 (11.11%)	0.41	8.00(0.28–449.98)
TT	2 (40.0%)	8 (44.44%)	0.92	2.00(0.10–69.78)
GT+TT	5 (80.0%)	10 (27.78%)	0.46	4.00(0.31–110.70)
Genotypes <i>CYP2B6</i> 516 G/T	Efavirenz + alcohol user N = 2 (%)	Efavirenz + alcohol nonuser N = 9 (%)	p-value	OR 95% CI
GG	2 (100%)	3 (33.33%)	1	Reference
GT	0	5 (55.56%)	NC	–
TT	0	1 (11.11%)	NC	–
GT+TT	0	6 (66.67%)	NC	–
Genotypes <i>CYP2B6</i> 516 G/T	Nevirapine + alcohol user N = 38 (%)	Nevirapine + alcohol nonuser N = 81 (%)	p-value	OR 95% CI
HIV patients without hepatotoxicity				
GG	8 (21.05%)	25 (30.86%)	1	Reference
GT	22 (57.89%)	28 (34.57%)	0.10	2.46 (0.84–7.31)
TT	8 (21.05%)	28 (34.57%)	0.10	0.89 (0.25–3.13)
GT+TT	30 (78.95%)	56 (69.14%)	0.37	1.67 (0.62–4.62)
Genotypes <i>CYP2B6</i> 516 G/T	Efavirenz + alcohol user N = 6 (%)	Efavirenz + alcohol nonuser N = 6 (%)	p-value	OR 95% CI
GG	3 (50.0%)	2(33.33%)	1	Reference
GT	2 (33.33%)	1 (16.67%)	0.57	1.33 (0.03–76.89)
TT	1 (16.67%)	3 (50.0%)	0.70	0.20 (0.00–7.34)
GT+TT	3 (50.0%)	4 (66.67%)	1.00	0.50 (0.02–8.76)

Note. N = number of subjects, (%) = frequency of genotypes. Presence of GG for GT, TT and GT+TT genotypes were taken as reference group for statistical analysis. NC = not calculable.

**TABLE 8** Multivariate analysis between HIV patients with and without hepatotoxicity

Variables	B	S.E.	df	p-value	OR (95%CI)
516GG			2	0.45	
516GT	–0.001	0.516	1	0.99	0.99 (0.36–2.74)
516 TT	–0.586	0.540	1	0.27	1.79 (0.62–5.17)
Age	–0.026	0.032	1	0.41	0.97 (0.91–1.03)
Sex	0.644	0.465	1	0.16	1.90 (0.76–4.73)
Tobacco user	0.196	0.608	1	0.747	1.217 (0.370–4.003)
Alcohol user	0.647	0.637	1	0.637	1.91 (0.766–4.739)
NNRTI drug user	–1.455	0.517	1	<b>0.005</b>	0.233 (0.085–0.643)
Baseline CD4 count	0.004	0.001	1	<b>0.001</b>	1.004 (1.002–1.007)

Note. *CYP2B6* 516G/T polymorphism, Age 18–50 year, sex, tobacco user, alcohol user, NNRTI drug user, Baseline CD4. Significant values (<0.05) represented in bold.

individuals with *CYP2B6*516TT genotype and alcohol and nevirapine usage are more prone to alcohol and drug-related hepatotoxicity.

Limitations of this study are as follows: (a) it could only define the association; (b) could not determine the causality; (c) could not estimate the plasma efavirenz and nevirapine level



in our subjects; (d) We had distributed a ratio of 1:4 for case controls. However, we could not complete matched enrollment in controls although our case–control ratio is around 1:3.

## 7 | CONCLUSION

The distribution of *CYP2B6* 516G>T polymorphism was not significantly different among HIV patients and healthy controls though, the prevalence of *CYP2B6* 516TT genotype was higher in patients with hepatotoxicity. *CYP2B6* genotype along with nevirapine and alcohol usage may influence the acquisition of ARV-associated hepatotoxicity and hepatotoxicity severity. The prevalence of *CYP2B6* 516TT genotype varied among different ethnic groups. Hence, further study of correlation of gene polymorphism with drug levels should be done in HIV patients on efavirenz 400 mg in a larger sample size. Finding of study may help to predict the acquisition of hepatotoxicity and also may influence the choice of drug to maintain the doses and its responses.

## ACKNOWLEDGMENT

We are grateful to all NARI-Clinical staff and community staff for the recruitment of subjects. We thank Asha Krishnaraj for helping in editing the manuscript. The study was supported by a research grant provided by NARI-ICMR India.

## CONFLICT OF INTEREST

None declared.

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## REFERENCES

- Bissell, D. M., Gores, G. J., Laskin, D. L., & Hoofnagle, J. H. (2001). Drug-induced liver injury: Mechanisms and test systems. *Hepatology*, 33(4), 1009–1013. <https://doi.org/10.1053/jhep.2001.23505>
- Cabrera, S. E., Santos, D., Valverde, M. P., Domínguez-Gil, A., González, F., Luna, G., & García, M. J. (2009). Influence of the cytochrome P450 2B6 genotype on population pharmacokinetics of efavirenz in human immunodeficiency virus patients. *Antimicrobial Agents and Chemotherapy*, 53(7), 2791–2798. <https://doi.org/10.1128/aac.01537-08>
- Carr, D. F., La Porte, C. J., Pirmohamed, M., Owen, A., & Cortes, C. P. (2010). Haplotype structure of *CYP2B6* and association with plasma efavirenz concentrations in a Chilean HIV cohort. *Journal of Antimicrobial Chemotherapy*, 65(9), 1889–1893. <https://doi.org/10.1093/jac/dkq260>
- Chen, J., Sun, J., Ma, Q., Yao, Y., Wang, Z., Zhang, L., ... Lu, H. (2010). *CYP2B6* polymorphism and nonnucleoside reverse transcriptase inhibitor plasma concentrations in Chinese HIV-infected patients. *Therapeutic Drug Monitoring*, 32(5), 573–578. <https://doi.org/10.1097/FTD.0b013e3181ea953c>
- Cho, J. Y., Lim, H. S., Chung, J. Y., Yu, K. S., Kim, J. R., Shin, S. G., & Jang, I. J. (2004). Haplotype structure and allele frequencies of *CYP2B6* in a Korean population. *Drug Metabolism and Disposition*, 32(12), 1341–1344. <https://doi.org/10.1124/dmd.104.001107>
- Deng, Y., Newman, B., Dunne, M. P., Silburn, P. A., & Mellick, G. D. (2004). Case-only study of interactions between genetic polymorphisms of *GSTM1*, *P1*, *T1* and *Z1* and smoking in Parkinson's disease. *Neuroscience Letters*, 366(3), 326–331. <https://doi.org/10.1016/j.neulet.2004.05.061>
- Desta, Z., Saussele, T., Ward, B., Bliedernicht, J., Li, L., Klein, K., ... Zanger, U. M. (2007). Impact of *CYP2B6* polymorphism on hepatic efavirenz metabolism in vitro. *Pharmacogenomics*, 8(6), 547–558. <https://doi.org/10.2217/14622416.8.6.547>
- Erickson, D. A., Mather, G., Trager, W. F., Levy, R. H., & Keirns, J. J. (1999). Characterization of the in vitro biotransformation of the HIV-1 reverse transcriptase inhibitor nevirapine by human hepatic cytochromes P-450. *Drug Metabolism and Disposition*, 27(12), 1488–1495.
- Gatanaga, H., Hayashida, T., Tsuchiya, K., Yoshino, M., Kuwahara, T., Tsukada, H., ... Oka, S. (2007). Successful efavirenz dose reduction in HIV type 1-infected individuals with cytochrome P450 2B6 \*6 and \*26. *Clinical Infectious Diseases*, 45(9), 1230–1237. <https://doi.org/10.1086/522175>
- Greenland, S. (1980). The effect of misclassification in the presence of covariates. *American Journal of Epidemiology*, 112(4), 564–569. <https://doi.org/10.1093/oxfordjournals.aje.a113025>
- Haas, D. W., Gebretsadik, T., Mayo, G., Menon, U. N., Acosta, E. P., Shintani, A., ... Wilkinson, G. R. (2009). Associations between *CYP2B6* polymorphisms and pharmacokinetics after a single dose of nevirapine or efavirenz in African Americans. *Journal of Infectious Diseases*, 199(6), 872–880. <https://doi.org/10.1086/597125>
- Haas, D. W., Smeaton, L. M., Shafer, R. W., Robbins, G. K., Morse, G. D., Labbe, L., ... Kim, R. B. (2005). Pharmacogenetics of long-term responses to antiretroviral regimens containing efavirenz and/or nelfinavir: An Adult Aids Clinical Trials Group Study. *Journal of Infectious Diseases*, 192(11), 1931–1942. <https://doi.org/10.1086/497610>
- Hiratsuka, M., Takekuma, Y., Endo, N., Narahara, K., Hamdy, S. I., Kishikawa, Y., ... Mizugaki, M. (2002). Allele and genotype frequencies of *CYP2B6* and *CYP3A5* in the Japanese population. *European Journal of Clinical Pharmacology*, 58(6), 417–421. <https://doi.org/10.1007/s00228-002-0499-5>
- Hofmann, M. H., Bliedernicht, J. K., Klein, K., Saussele, T., Schaeffeler, E., Schwab, M., & Zanger, U. M. (2008). Aberrant splicing caused by single nucleotide polymorphism c.516G>T [Q172H], a marker of *CYP2B6*\*6, is responsible for decreased expression and activity of *CYP2B6* in liver. *Journal of Pharmacology and Experimental Therapeutics*, 325(1), 284–292. <https://doi.org/10.1124/jpet.107.133306>
- Lang, T., Klein, K., Fischer, J., Nüssler, A. K., Neuhaus, P., Hofmann, U., ... Zanger, U. M. (2001). Extensive genetic polymorphism in the human *CYP2B6* gene with impact on expression and function in human liver. *Pharmacogenetics*, 11(5), 399–415.
- Lindfelt, T., O'Brien, J., Song, J. C., Patel, R., & Winslow, D. L. (2010). Efavirenz plasma concentrations and cytochrome 2B6

- polymorphisms. *Annals of Pharmacotherapy*, 44(10), 1572–1578. <https://doi.org/10.1345/aph.1P141>
- Mahungu, T., Smith, C., Turner, F., Egan, D., Youle, M., Johnson, M., ... Owen, A. (2009). Cytochrome P450 2B6 516G>T is associated with plasma concentrations of nevirapine at both 200 mg twice daily and 400 mg once daily in an ethnically diverse population. *HIV Medicine*, 10(5), 310–317. <https://doi.org/10.1111/j.1468-1293.2008.00689.x>
- Mehlotra, R. K., Cheruvu, V. K., Blood Zikursh, M. J., Benish, R. L., Lederman, M. M., Salata, R. A., ... Rodriguez, B. (2011). Chemokine (C-C motif) receptor 5–2459 genotype in patients receiving highly active antiretroviral therapy: Race-specific influence on virologic success. *Journal of Infectious Diseases*, 204(2), 291–298. <https://doi.org/10.1093/infdis/jir262>
- Motsinger, A. A., Ritchie, M. D., Shafer, R. W., Robbins, G. K., Morse, G. D., Labbe, L., ... Haas, D. W. (2006). Multilocus genetic interactions and response to efavirenz-containing regimens: An Adult AIDS Clinical Trials Group Study. *Pharmacogenetics and Genomics*, 16(11), 837–845. <https://doi.org/10.1097/01.fpc.0000230413.97596.fa>
- Nagpal, M., Tayal, V., Kumar, S., & Gupta, U. (2010). Adverse drug reactions to antiretroviral therapy in AIDS patients at a tertiary care hospital in India: A prospective observational study. *Indian Journal of Medical Sciences*, 64(6), 245–252. <https://doi.org/10.4103/0019-5359.99597>
- Nyakutira, C., Röshammar, D., Chigutsa, E., Chonzi, P., Ashton, M., Nhachi, C., & Masimirembwa, C. (2008). High prevalence of the CYP2B6 516→T (\*6) variant and effect on the population pharmacokinetics of efavirenz in HIV/AIDS outpatients in Zimbabwe. *European Journal of Clinical Pharmacology*, 64(4), 357–365. <https://doi.org/10.1007/s00228-007-0412-3>
- Penzak, S. R., Kabuye, G., Mugenyi, P., Mbamanya, F., Natarajan, V., Alfaro, R. M., ... Masur, H. (2007). Cytochrome P450 2B6 (CYP2B6) G516T influences nevirapine plasma concentrations in HIV-infected patients in Uganda. *HIV Medicine*, 8(2), 86–91. <https://doi.org/10.1111/j.1468-1293.2007.00432.x>
- Putthanakit, T., Tanpaiboon, P., Aupibul, L., Cressey, T. R., & Sirisanthana, V. (2009). Plasma efavirenz concentrations and the association with CYP2B6–516G>T polymorphism in HIV-infected Thai children. *Antiviral Therapy*, 14(3), 315–320.
- Ramachandran, G., Ramesh, K., Hemanth, A. K., Jagan, I., Vasantha, M., Padmapriyadarsini, C., ... Swaminathan, S. (2009). Association of high T allele frequency of CYP2B6 G516T polymorphism among ethnic south Indian HIV-infected patients with elevated plasma efavirenz and nevirapine. *Journal of Antimicrobial Chemotherapy*, 63(4), 841–843. <https://doi.org/10.1093/jac/dkp033>
- Reisler, R., Servoss, J., & Sherman, K. (2001). Incidence of hepatotoxicity and mortality in 21 adult antiretroviral treatment trials. 1st IAS Conference on HIV Pathogenesis and Treatment. Buenos Aires.
- Ribaudo, H. J., Haas, D. W., Tierney, C., Kim, R. B., Wilkinson, G. R., Gulick, R. M., ... Acosta, E. P.; Adult AIDS Clinical Trials Group Study. (2006). Pharmacogenetics of plasma efavirenz exposure after treatment discontinuation: An adult AIDS clinical trials group study. *Clinical Infectious Diseases*, 42(3), 401–407. <https://doi.org/10.1086/499364>
- Ribaudo, H. J., Liu, H., Schwab, M., Schaeffeler, E., Eichelbaum, M., Motsinger-Reif, A. A., ... Haas, D. W. (2007). Effect of CYP2B6, ABCB1, and CYP3A5 polymorphisms on efavirenz pharmacokinetics and treatment response: An AIDS Clinical Trials Group study. *Journal of Infectious Diseases*, 202, 717–722. <https://doi.org/10.1086/655470>
- Rodriguez-Novoa, S., Barreiro, P., Rendón, A., Jiménez-Nacher, I., González-Lahoz, J., & Soriano, V. (2005). Influence of 516G>T polymorphisms at the gene encoding the CYP450-2B6 isoenzyme on efavirenz plasma concentrations in HIV infected subjects. *Clinical Infectious Diseases*, 40(9), 1358–1361. <https://doi.org/10.1086/429327>
- Rotger, M., Tegude, H., Colombo, S., Cavassini, M., Furrer, H., Decosterd, L., ... Zanger, U. M. (2007). Predictive value of known and novel alleles of CYP2B6 for efavirenz plasma concentrations in HIV-infected individuals. *Clinical Pharmacology and Therapeutics*, 81(4), 557–566. <https://doi.org/10.1038/sj.clpt.6100072>
- Sadiq, S. T., Fredericks, S., Khoo, S. H., Rice, P., & Holt, D. W. (2005). Efavirenz detectable in plasma 8 weeks after stopping therapy and subsequent development of non-nucleoside reverse transcriptase inhibitor-associated resistance. *AIDS*, 19(15), 1716–1717. <https://doi.org/10.1097/01.aids.0000186828.99032.60>
- Saitoh, A., Sarles, E., Capparelli, E., Aweeka, F., Kovacs, A., Burchett, S. K., ... Spector, S. A. (2007). CYP2B6 genetic variants are associated with nevirapine and clinical response in HIV-1 infected children. *AIDS*, 21(16), 2191–2199. <https://doi.org/10.1097/qad.0b013e3282ef9695>
- Samet, J. H., Cheng, D. M., Libman, H., Nunes, D. P., Alperen, J. K., & Saitz, R. (2007). Alcohol consumption and HIV disease progression. *Journal of Acquired Immune Deficiency Syndromes*, 46(2), 194–199. <https://doi.org/10.1097/QAI.0b013e318142aabb>
- Schipani, A., Wyen, C., Mahungu, T., Hendra, H., Egan, D., Siccardi, M., ... Back, D. J.; German Competence Network for HIV/AIDS. (2011). Integration of population pharmacokinetics and pharmacogenetics: An aid to optimal nevirapine dose selection in HIV-infected individuals. *Journal of Antimicrobial Chemotherapy*, 66(6), 1332–1339. <https://doi.org/10.1093/jac/dkr087>
- Servoss, J. C., Kitch, D. W., Andersen, J. W., Reisler, R. B., Chung, R. T., & Robbins, G. K. (2006). Predictors of antiretroviral-related hepatotoxicity in the adult AIDS Clinical Trial Group (1989–1999). *Journal of Acquired Immune Deficiency Syndromes*, 43(3), 320–323. <https://doi.org/10.1097/01.qai.0000243054.58074.59>
- To, K. W., Liu, S. T., Cheung, S. W., Chan, D. P., Chan, R. C., & Lee, S. S. (2009). Pharmacokinetics of plasma efavirenz and CYP2B6 polymorphism in southern Chinese. *Therapeutic Drug Monitoring*, 31(4), 527–530. <https://doi.org/10.1097/FTD.0b013e3181ad74a4>
- Tong, K., He, M. L., Lin, C. K., Guo, L., Kung, H. F., Sung, J. J., & Lee, S. S. (2006). The implications of a high allelic frequency of CYP2B6 G516T in ethnic Chinese persons. *Clinical Infectious Diseases*, 43(4), 541–542. <https://doi.org/10.1086/508621>
- Van Dyke, R. B., Wang, L., & Williams, P. L.; Pediatric AIDS Clinical Trials Group 219C Team. (2008). Toxicities associated with dual nucleoside reverse-transcriptase inhibitor regimens in HIV-infected children. *The Journal of Infectious Diseases*, 198(11), 1599–1608. <https://doi.org/10.1086/593022>
- van Leth, F., Phanuphak, P., Ruxrungtham, K., Baraldi, E., Miller, S., Gazzard, B., ... 2NN Study team. (2004). Comparison of first-line antiretroviral therapy with regimens including nevirapine, efavirenz, or both drugs, plus stavudine and lamivudine: A randomized open-label trial, the 2NN Study. *The Lancet*, 363(9417):1253–1263. [https://doi.org/10.1016/s0140-6736\(04\)15997-7](https://doi.org/10.1016/s0140-6736(04)15997-7)
- Ward, B. A., Gorski, J. C., Jones, D. R., Hall, S. D., Flockhart, D. A., & Desta, Z. (2003). The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: Implication for HIV/AIDS therapy and utility of efavirenz

- as a substrate marker of CYP2B6 catalytic activity. *Journal of Pharmacology and Experimental Therapeutics*, 306(1), 287–300. <https://doi.org/10.1124/jpet.103.049601>
- Watanabe, T., Sakuyama, K., Sasaki, T., Ishii, Y., Ishikawa, M., Hirasawa, N., & Hiratsuka, M. (2010). Functional characterization of 26 CYP2B6 allelic variants (CYP2B6.2–CYP2B6.28, except CYP2B6.22). *Pharmacogenetics and Genomics*, 20(7), 459–462. <https://doi.org/10.1097/FPC.0b013e32833bba0e>
- Wyen, C., Hendra, H., Vogel, M., Hoffmann, C., Knechten, H., Brockmeyer, N. H., ... Owen, A.; German Competence Network for HIV/AIDS. (2008). Impact of CYP2B6 983T>C polymorphism on non-nucleoside reverse transcriptase inhibitor plasma concentrations in HIV-infected patients. *Journal of Antimicrobial Chemotherapy*, 61(4), 914–918. <https://doi.org/10.1093/jac/dkn029>
- Xu, B. Y., Guo, L. P., Lee, S. S., Dong, Q. M., Tan, Y., Yao, H., ... He, M. L. (2007). The genetic variability of CYP2B6 polymorphisms in four southern Chinese populations. *World Journal of Gastroenterology*, 13(14), 2100–2103. <https://doi.org/10.3748/wjg.v13.i14.2100>
- Zanger, U. M., Klein, K., Saussele, T., Bliedernicht, J., Hofmann, M. H., & Schwab, M. (2007). Polymorphic CYP2B6: Molecular mechanisms and emerging clinical significance. *Pharmacogenomics*, 8(7), 743–759. <https://doi.org/10.2217/14622416.8.7.743>
- Zhang, H., Sridar, C., Kenaan, C., Amunugama, H., Ballou, D. P., & Hollenberg, P. F. (2011). Polymorphic variants of cytochrome P450 2B6 (CYP2B6.4–CYP2B6.9) exhibit altered rates of metabolism for bupropion and efavirenz: A charge-reversal mutation in the K139E variant (CYP2B6.8) impairs formation of a functional P450-reductase complex. *Journal of Pharmacology and Experimental Therapeutics*, 338(3), 803–809. <https://doi.org/10.1124/jpet.111.183111>

**How to cite this article:** Singh H, Lata S, Dhole TN, Gangakhedkar RR. Occurrence of CYP2B6 516G>T polymorphism in patients with ARV-associated hepatotoxicity. *Mol Genet Genomic Med*. 2019;7:e598. <https://doi.org/10.1002/mgg3.598>