Prevalence of ABCC3-1767G/A polymorphism among patients with antiretroviral-associated hepatotoxicity

HariOm Singh1 | Sonam Lata1 | Ranjana Choudhari2 | Tapan N. Dhole3

1Department of Molecular Biology, National AIDS Research Institute, Pune, India
2Department of Clinical Epidemiology, National Institute of Occupational Health, Ahmedabad, India
3Department of Microbiology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India

Abstract

Background: Plasma concentrations of antiretrovirals (ARVs) regimens have considerably varied in individuals of human immunodeficiency virus (HIV) because of variations in the expression of drug-metabolizing and transporter genes. Transporter genes play an important role in the disposition of drugs. Polymorphism in transporter gene (ABCC3) affects the MRP3 expression and varies the treatment outcome.

Method: We examined the polymorphism of ABCC3-1767G/A gene in a total of 165 HIV patients (out of 165 HIV patients, 34 were with and 131 were without hepatotoxicity) and 156 healthy individuals using the polymerase chain reaction–restriction fragment length polymorphism method.

Results: In univariate analysis, we found a decreased prevalence of ABCC3 1767GA, 1767GA+AA genotypes, and 1767A allele in patients with hepatotoxicity as compared to patients without hepatotoxicity (23.5% vs. 28.2% and 23.5% vs. 30.53%; 11.76% vs. 16.41%), while a higher prevalence of 1767AA genotype was observed in HIV patients in comparison with healthy controls (2.3% vs. 1.3%, odds ratio [OR] = 1.71, 95% confidence interval [CI]: 0.23–15.03, p = .89). The frequency of ABCC3-1767AA genotype was dispersed higher in individuals with early and advanced HIV disease stage in comparison with healthy controls (5.3% vs. 1.3%, OR = 4.73, p = .70; 8.9% vs. 1.3%, OR = 1.89, p = .91). A higher occurrence of ABCC3-1767AA genotype was found in tobacco using HIV patients receiving nevirapine irrespective of their hepatotoxicity status as compared to nonusers (4.7% vs. 1.1%, OR = 4.28, p = .52). The distribution of ABCC3-1767GA genotype was dispersed higher in nevirapine receiving HIV patients with hepatotoxicity compared with nonusers (30.4% vs. 9.1%, OR = 3.34, p = .77). In multivariate analysis, HIV patients receiving nevirapine and with hepatotoxicity was found to have a significant risk for severity of hepatotoxicity (OR = 4.56, 95% CI: 1.60–12.99, p = .004).

Conclusion: ABCC3 1767GA polymorphism was not significantly associated with susceptibility to ARV-associated hepatotoxicity, although ABCC3 1767AA genotype designated a risk for acquisition of hepatotoxicity and advancement of the disease. Nevirapine usage emerged as an independent risk factor for hepatotoxicity severity.
1 | INTRODUCTION

Antiretroviral therapy (ART) is a basis for the treatment in human immunodeficiency virus (HIV) infection. However, long-term efficacy and toxicity are the major challenges when selecting an ART regimen for the treatment of HIV. Liver is a primary organ for drug metabolism and detoxification in the body in addition to being a major target of drug toxicity (Bandara & Kennedy, 2002). High levels of antiretrovirals (ARVs) in HIV patients may cause several adverse drug reactions (ADR) like liver toxicity. Hepatotoxicity is one of the common ADR leading to the interruptions in the treatment in HIV patients (Van Dyke, Wang, & Williams, 2008). Usage of nevirapine-based ART was associated with a higher incidence of ARV-associated hepatotoxicity toxicity than efavirenz (Van Leth, Phanuphak, & Ruxrunghath, 2004). Reisler et al. (2001) reported that the incidence of severe hepatotoxicity was 10.8% in the efavirenz-treated group and 8.9% in the nevirapine-treated group. The incidence rate of nevirapine-induced hepatotoxicity was 3.19% in India (Nagpal, Tayal, Kumar, & Gupta, 2010).

Antiretroviral therapy modifies the activity and expression of drug transporters. This could alter the drug absorption, elimination, and distribution and thereby affect the access to the target site. Multidrug resistance-related proteins (MRP/ABCC) play an important role in elimination of numerous drugs including non-nucleoside reverse transcriptase inhibitors (NNRTI) (Borst, Evers, & Kool, 2000). ABCC3 plays a role in toxicological defense; it eliminates a range of (toxic) anions from hepatocytes. Polymorphism (−1767G/A) of ABCC3 gene affects the expression levels, which leads to toxicological effects. Till date, the prevalence of promoter region polymorphism (−1767G/A) of ABCC3 gene among patients with ARV-associated hepatotoxicity has not been studied worldwide. Hence, we investigated the prevalence of ABCC3-1767G/A polymorphism in two groups of HIV patients, made on the basis of the presence or absence of ARV-associated hepatotoxicity.

2 | MATERIAL AND METHODS

2.1 | Subjects

The present case–control study was undertaken from October 2013 to March 2016, at the outpatient HIV clinics of the National AIDS Research Institute, Pune. The study included three groups, one consisting of 34 patients with hepatotoxicity (Grade III/IV) under NNRTI containing ART regimen, 131 HIV patients without hepatotoxicity confirmed by liver function tests (LFT), and 156 age-matched healthy controls. Patients with hepatotoxicity due to other causes such as hepatitis B, hepatitis C, tuberculosis, and concurrent untreated opportunistic infections, immune reconstitution syndrome, and receiving any other known hepatotoxic drugs were excluded from the study. Control group comprised of age-matched, one fifty-six healthy individuals (two or more than two persons belonging to the same family were excluded in order to find out the true prevalence of genetic variation), who were free from HIV (serum-negative on HIV-ELISA test), hepatitis B, hepatitis C, and tuberculosis infections. ELISA for hepatitis C and HBsAg testing was performed using Ortho HCV ELISA test system and Murex HBsAg confirmatory (DiaSorin) ELISA, respectively. The study was approved by
the institutional ethics committee, and written informed consent was obtained from all eligible participants.

Clinical research proforma/data collection was done using pretested, prevalidated questionnaire, personal interviews, and review of existing case records. Environmental exposures such as tobacco use and alcohol usage/intake for each subject were also recorded in the questionnaire.

Liver function test was done to evaluate the status of liver enzymes. Total bilirubin >3.22 mg/ml, SGOT >93.8 U/ml, SGPT >229.5 U/ml, and alkaline phosphatase >550.8 U/ml parameters defined the cases with hepatotoxicity in male gender, whereas total bilirubin >3.22 mg/ml, SGOT >163.2 U/ml, SGPT >173.4 U/ml, and alkaline phosphatase >550.8 U/ml formed the case definition for hepatotoxicity in female. Total bilirubin <1.24 mg/ml, SGOT <32 U/ml, SGPT <34 U/ml, and alkaline phosphatase <108 U/ml were the criteria for labeling/recruiting HIV-infected but without hepatotoxicity group for both genders. Estimation of CD4 count was done by fluorescently activated cell sorter. CD4 status was used to classify patients into different subgroups, representing the severity stages of the HIV infection. CD4 ranges from <200 cells/mm³ as defined as an advanced stage, 201–350 cells/mm³ as an intermediate stage, and >350 cells/mm³ onward as an early stage of HIV infection.

2.2 | DNA extraction

A peripheral blood sample of 2 ml was obtained and stored at −70°C prior to extraction of genomic DNA, which was done from peripheral blood leukocyte pellet using the QIAamp DNA Mini Kit (QIAGEN Str. 1 40724) according to the protocol given by the manufacturer of Mini Kit.

2.3 | Genotyping

The genotyping of ABCC3-1767GA polymorphism was done by polymerase chain reaction–restriction fragment length polymorphism in study subjects. The primer used for amplification of ABCC3-1767GA polymorphism was taken from the study carried out by Fukuda et al. (2010). PCR was performed in a total volume of 20 μl with 20 pmol of each primer, genomic DNA (100–150 ng), 10 mM deoxynucleotide triphosphates, PCR buffer containing 100 mM Tris-HCl, pH 8.6, 50 mM KCl, 1.5 mM MgCl₂, and 1.5 units of Taq polymerase (Bangalore Genei, India). The reaction conditions for ABCC3-1767GA were as follows: initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 57°C for 30 s, extension at 72°C for 45 s, and final extension at 72°C for 10 min. Amplified product of ABCC3 was digested using restriction enzyme BsmAI (Fermentas Inc.). ABCC3-1767GA polymorphism was genotyped in 15% polyacrylamide gel using molecular weight markers and visualized after staining with ethidium bromide. Based on sequences and location of SNP, genotypes of ABCC3 were assigned as follows: for ABCC3: 281bp for −1767GG, 281 bp + 241 bp + 40 bp for 1767GA, and 241 bp + 40 bp for GG 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 regenotyped by another trained laboratory personnel to rule out discrepancy in genotyping reporting. Ten percent of samples were sequenced to assess the genotyping error.

2.4 | Data analysis

The age variable was expressed as mean ± standard deviation (SD). The deviation from Hardy–Weinberg equilibrium in controls was analyzed using chi-square 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-square statistic (Fisher’s exact test for theoretical cell size <5). Tobacco, alcohol usages and genotypes interactions 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.

3 | RESULTS

The study consisted of a total of 165 HIV patients of which 34 patients had hepatotoxicity, 131 were without hepatotoxicity, and 156 were healthy controls. The mean age (years ± SD) of patients with hepatotoxicity, patients without hepatotoxicity, and healthy controls was 40.12 years ± 4.31, 39.42 years ± 3.42, and 37 years ± 4.35, respectively. The demographic profile of HIV patients with, without hepatotoxicity and healthy controls is shown in Table 1.

3.1 | Association of genotype and phenotype

3.1.1 | ABCC3-1767GA polymorphism and patients with ARV-associated hepatotoxicity

The genotype and allele frequency of ABCC3-1767GA polymorphism in between HIV patients with and without hepatotoxicity and between HIV patients with hepatotoxicity and healthy controls are shown in Table 2. The distributions of
ABCC3-1767GA polymorphism has followed the Hardy–Weinberg equilibrium \((p = .13)\) in healthy controls. The distributions of \(ABCC3\)-1767GA polymorphism in HIV patients without hepatotoxicity and healthy controls are presented in Table 3. The occurrence of \(ABCC3\)-1767GG, 1767GA, 1767GA+AA genotypes and \(ABCC3\)-1767A allele found almost similar among HIV patients without hepatotoxicity and healthy controls (69.5% vs. 67.7%; 28.2% vs. 32.1%; 30.53% vs. 33.33%; 16.41% vs. 17.30%, respectively). The prevalence of \(-1767AA\) genotype was higher in HIV patients without hepatotoxicity as compared to healthy controls (2.3% vs. 1.3%, \(OR = 1.71, 95\% CI: 0.23–15.03, p = .89\)).

### 3.1.3 Association of genotype and HIV disease stages

The occurrence of \(ABCC3\)-1767GA genotype was higher in individuals with early HIV disease stage compared with healthy controls (57.9% vs. 67.7%). The incidence of \(ABCC3\)-1767AA genotype observed to be higher in individuals with early and advanced HIV disease stage in comparison with healthy controls (5.3% vs. 1.3%, \(OR = 4.73, 95\% CI: 0.0–76.30, p = .70\); 8.9% vs. 1.3%, \(OR = 1.89, 95\% CI: 0.18–19.42, p = .91\)) (Table 4).

### 3.1.4 Association of genotype–environment interaction

In patients with hepatotoxicity, \(ABCC3\)-1767GA genotype was underrepresented in tobacco using as compared with tobacco nonusers (14.3% vs. 25.9%, \(OR = 0.55, 95\% CI: 0.08–3.77, p = .46\)), while \(ABCC3\)-1767AA genotype was overrepresented in tobacco using HIV patients without hepatotoxicity compared with nonusers (4.7% vs. 1.1%, \(OR = 4.28, 95\% CI: 0.29–124.5, p = .52\)) (Table 5).

The occurrence of \(ABCC3\)-1767GA was almost similar in tobacco using HIV patients without hepatotoxicity compared with nonusers (27.9% vs. 28.4%, \(OR = 1.03, 95\% CI: 0.42–2.50, p = .88\)). In both the groups of HIV patients (with and without hepatotoxicity), almost similar results were obtained while comparing the distribution of \(ABCC3\)-1767GA polymorphism between alcohol consuming and nonalcoholics (Table 6).

The distribution of \(ABCC3\)-1767GA polymorphism in HIV patients with hepatotoxicity, who are further subclassified on the basis of the receiving of two ART drugs, namely nevirapine and efavirenz, is shown in Table 7. Similar drug groupwise frequency distribution of \(ABCC3\)-1767GA polymorphism in HIV patients without hepatotoxicity is also shown in Table 7. In both the groups of HIV patients (with and without hepatotoxicity),

### 3.1.2 \(ABCC3\)-1767G/A polymorphism and HIV patients without hepatotoxicity

We have calculated the Hardy–Weinberg equilibrium in healthy control population. We found that the distribution of
The incidences of ABCC3-1767GA genotype were observed to be increased in nevirapine users in comparison with efavirenz users (30.4% vs. 9.1%, OR = 3.34, 95% CI: 0.46–23.96, \( p = .22 \); 29.4% vs. 16.7%, OR = 0.73 (0.30–1.77)).

While in HIV patients without hepatotoxicity, the occurrence of ABCC3-1767AA genotype was decreased in nevirapine users as compared to efavirenz users (0.8% vs. 16.7%, OR = 0.05, 95% CI: 0.00–0.80, \( p = .02 \)).

### Table 2

| Genotype ABCC3-1767G/A | HIV patients with hepatotoxicity \( N = 34 \) (%) | HIV patients without hepatotoxicity \( N = 131 \) (%) | \( p \)-Value | OR (95% CI) |
|------------------------|------------------------------------------------|------------------------------------------------|---------------|------------|
| GG                     | 26 (76.5%)                                    | 91 (69.5%)                                    | —             | 1 (Reference) |
| GA                     | 8 (23.5%)                                     | 37 (28.2%)                                    | 0.48          | 0.73 (0.30–1.77) |
| AA                     | 0 (0.0%)                                      | 3 (2.3%)                                      | NS            | —          |
| GA+AA                  | 8 (23.5%)                                     | 40 (30.53%)                                   | 0.55          | 0.70 (0.27–1.80) |

| ABCC3-1767G/A Allele | HIV patients with hepatotoxicity \( N = 68 \) (%) | Healthy control \( N = 262 \) (%) | \( p \)-Value | OR (95% CI) |
|----------------------|------------------------------------------------|----------------------------------|---------------|------------|
| G                    | 60 (88.23%)                                    | 219 (83.58%)                    | —             | 1 (Reference) |
| A                    | 8 (11.76%)                                     | 43 (16.41%)                     | .44           | 0.68 (0.28–1.60) |

| Genotype ABCC3-1767G/A | HIV patients with hepatotoxicity \( N = 34 \) (%) | Healthy control \( N = 156 \) (%) | \( p \)-Value | OR (95% CI) |
|------------------------|------------------------------------------------|---------------------------------|---------------|------------|
| GG                     | 26 (76.5%)                                    | 104 (67.7%)                    | —             | 1 (Reference) |
| GA                     | 8 (23.5%)                                     | 50 (32.1%)                     | .50           | 0.70 (0.25–1.93) |
| AA                     | 0 (0.0%)                                      | 2 (1.3%)                       | NS            | —          |
| GA+AA                  | 8 (23.5%)                                     | 52 (33.33%)                    | .36           | 0.62 (0.24–1.55) |

| ABCC3-1767G/A Allele | HIV patients with hepatotoxicity \( N = 68 \) (%) | Healthy control \( N = 312 \) (%) | \( p \)-Value | OR (95% CI) |
|----------------------|------------------------------------------------|---------------------------------|---------------|------------|
| G                    | 60 (88.23%)                                    | 258 (83.22%)                  | —             | 1 (Reference) |
| A                    | 8 (11.76%)                                     | 54 (17.30%)                   | .34           | 0.64 (0.26–1.48) |

Note: \( N \), total number of HIV patients with hepatotoxicity (34), HIV patients without hepatotoxicity (131), and healthy controls (156). Odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype/allele (GG genotype and G allele for ABCC3-1767G/A) with other genotypes/alleles.

Abbreviation: HIV, human immunodeficiency virus.

### Table 3

| Genotype ABCC3-1767G/A | HIV patients \( N = 131 \) (%) | Healthy Control \( N = 156 \) (%) | \( p \)-Value | OR (95% CI) |
|------------------------|--------------------------------|---------------------------------|---------------|------------|
| GG                     | 91 (69.5%)                    | 104 (67.7%)                    | —             | 1 (Reference) |
| GA                     | 37 (28.2%)                    | 50 (32.1%)                     | .98           | 0.99 (0.53–1.83) |
| AA                     | 3 (2.3%)                      | 2 (1.3%)                       | .89           | 1.71 (0.23–15.03) |
| GA+AA                  | 40 (30.53%)                   | 52 (33.33%)                    | .70           | 0.88 (0.52–1.49) |

| ABCC3-1767G/A Allele | HIV patients \( N = 262 \) (%) | Healthy Control \( N = 310 \) (%) | \( p \)-Value | OR (95% CI) |
|----------------------|--------------------------------|---------------------------------|---------------|------------|
| G                    | 219 (83.58%)                  | 258 (83.22%)                   | —             | 1 (Reference) |
| A                    | 43 (16.41%)                   | 54 (17.30%)                    | .86           | 0.94 (0.59–1.49) |

Note: \( N \), total number of HIV patients (34) and healthy controls (155). Odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype/allele (GG genotype and G allele for ABCC3-1767G/A) with other genotypes/alleles.

Abbreviation: HIV, human immunodeficiency virus.
In HIV patients without hepatotoxicity, the prevalence of ABCC3-1767AA genotype was reduced in alcohol + nevirapine and alcohol + efavirenz consumers as compared to nonuser (7.89% vs. 14.82%, OR = 0.47, 95% CI: 0.09–2.16, \( p = .44 \); 16.67% vs. 83.33%, OR = 0.04, 95% CI: 0.0–1.29, \( p = .08 \)) (Table 8).

### Table 4: Frequency distribution of ABCC3-1767G/A genotypes in different HIV disease stages and healthy controls

| Genotype ABCC3-1767G/A | Healthy controls \( N = 156 \) (%) | Early HIV disease stage \( N = 19 \) (%) | Intermediate HIV disease stage \( N = 33 \) (%) | Advanced HIV disease stage \( N = 79 \) (%) |
|------------------------|------------------------------------|---------------------------------|---------------------------------|---------------------------------|
| GG                     | 104 (67.7%)                        | 11 (57.9%)                      | 25 (75.8%)                      | 55 (69.6%)                      |
| GA                     | 50 (32.1%)                         | 7 (36.8%)                       | 8 (24.2%)                       | 22 (27.8%)                      |
| AA                     | 2 (1.3%)                           | 1 (5.3%)                        | 0 (0.0%)                        | 2 (8.9%)                        |

Note: (%), frequency of subjects, odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype (GG genotype and G allele for ABCC3-1767G/A) with other genotypes. HIV, human immunodeficiency virus; \( N \), number of subjects.

### Table 5: Frequency distribution of ABCC3-1767G/A genotypes in tobacco using HIV patients with and without hepatotoxicity

#### HIV patients with hepatotoxicity

| Genotype ABCC3-1767G/A | Tobacco user \( N = 7 \) (%) | Tobacco nonuser \( N = 27 \) (%) | \( p \)-Value | OR (95% CI) |
|------------------------|-------------------------------|-------------------------------|--------------|--------------|
| GG                     | 6 (85.7%)                     | 20 (74.1%)                   | —            | 1 (Reference) |
| GA                     | 1 (14.3%)                     | 7 (25.9%)                    | .46          | 0.55 (0.08–3.77) |
| AA                     | 0 (0.0%)                      | 0 (0.0%)                     | —            | —            |

#### HIV patients without hepatotoxicity

| Genotype ABCC3-1767G/A | Tobacco user \( N = 43 \) (%) | Tobacco nonuser \( N = 88 \) (%) | \( p \)-Value | OR (95% CI) |
|------------------------|-------------------------------|-------------------------------|--------------|--------------|
| GG                     | 29 (67.4%)                   | 62 (70.5%)                   | —            | 1 (Reference) |
| GA                     | 12 (27.9%)                   | 25 (28.4%)                   | .88          | 1.03 (0.42–2.50) |
| AA                     | 2 (4.7%)                     | 1 (1.1%)                     | .52          | 4.28 (0.29–124.5) |

Note: (%), frequency of subjects, odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype (GG genotype and G allele for ABCC3-1767G/A) with other genotypes. HIV, human immunodeficiency virus; \( N \), number of subjects.

### Table 6: Frequency distribution of ABCC3-1767G/A genotypes in alcohol using HIV patients with and without hepatotoxicity

#### HIV patients with hepatotoxicity

| Genotype ABCC3-1767G/A | Alcohol user \( N = 7 \) (%) | Alcohol nonuser \( N = 27 \) (%) | \( p \)-Value | OR (95% CI) |
|------------------------|-------------------------------|-------------------------------|--------------|--------------|
| GG                     | 6 (85.7%)                     | 20 (74.1%)                   | —            | 1 (Reference) |
| GA                     | 1 (14.3%)                     | 7 (25.9%)                    | .46          | 0.55 (0.08–3.77) |
| AA                     | 0 (0.0%)                      | 0 (0.0%)                     | —            | —            |

#### HIV patients without hepatotoxicity

| Genotype ABCC3-1767G/A | Alcohol user \( N = 44 \) (%) | Alcohol nonuser \( N = 87 \) (%) | \( p \)-Value | OR (95% CI) |
|------------------------|-------------------------------|-------------------------------|--------------|--------------|
| GG                     | 29 (65.9%)                   | 62 (71.3%)                   | —            | 1 (Reference) |
| GA                     | 13 (29.5%)                   | 24 (27.6%)                   | .88          | 1.16 (0.48–2.79) |
| AA                     | 2 (4.5%)                     | 1 (1.1%)                     | .52          | 4.28 (0.29–124.55) |

Note: (%), frequency of subjects, odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype (GG genotype and G allele for ABCC3-1767G/A) with other genotypes. HIV, human immunodeficiency virus; \( N \), number of subjects.
3.2 Risk factors of ARV-associated hepatotoxicity: multivariate logistic regression analysis

Relationships of age, sex, tobacco, alcohol, baseline CD4 counts, and *ABCC3*-1767G/A polymorphism with ARV-associated hepatotoxicity were explored by multivariate logistic regression analysis. *ABCC3*-1767G/A polymorphism, age, sex, tobacco, alcohol usage, and baseline CD4 counts were not associated with susceptibility to ARV-associated hepatotoxicity. While comparing between HIV patients with and without hepatotoxicity, nevirapine showed a significant risk for occurrence of severity of hepatotoxicity (OR = 4.56, 95% CI: 1.60–12.99, *p* = .004) (Table 9).

### TABLE 7 Frequency distribution of *ABCC3*-1767G/A genotypes in HIV patients receiving NNRTI regimen with and without hepatotoxicity

| Genotype *ABCC3*-1767G/A | Nevirapine users *N* = 23 (%) | Efavirenz users *N* = 11 (%) | p-Value | OR (95% CI) |
|---------------------------|-------------------------------|-----------------------------|---------|-------------|
| GG                        | 16 (69.6%)                    | 10 (90.9%)                  | —       | 1 (Reference) |
| GA                        | 7 (30.4%)                     | 1 (9.1%)                    | .22     | 3.34 (0.46–23.96) |
| AA                        | 0 (0.0%)                      | 0 (0.0%)                    | —       | —            |

| Genotype *ABCC3*-1767G/A | Nevirapine users *N* = 119 (%) | Efavirenz users *N* = 12 (%) | p-Value | OR (95% CI) |
|---------------------------|--------------------------------|-----------------------------|---------|-------------|
| GG                        | 83 (69.7%)                    | 8 (66.7%)                   | —       | 1 (Reference) |
| GA                        | 35 (29.4%)                    | 2 (16.7%)                   | .77     | 1.69 (0.31–12.15) |
| AA                        | 1 (0.8%)                      | 2 (16.7%)                   | .02     | 0.05 (0.00–0.80) |

Note: (%), frequency of subjects, odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype (GG genotype and G allele for *ABCC3*-1767G/A) with other genotypes. HIV, human immunodeficiency virus; *N*, number of subjects; NNRTI, non-nucleoside reverse transcriptase inhibitors; NS, not significant.

4 DISCUSSION

Antiretrovirals alter the activity and expression of active drug transporters, which in turn can affect the drug absorption, elimination, and distribution, thereby playing an important role in the treatment outcome. NNRTI (delavirdine, efavirenz, and nevirapine) interact with MRP3 in vitro. MRP3 proteins are one of the main types of transporter proteins, which play an important role in the drug transport of major drugs including efavirenz (Zhou, Di, & Chan, 2008). The genetic variation may affect the global response to treatment or causing adverse drug events. Large interindividual variabilities in the expression of transporters may lead to inappropriate drug concentrations, causing drug toxicity or insufficient therapeutic effects in the liver. Evidence has revealed that variabilities in the expression and activities of some transporters affect pharmacokinetics as well as pharmacological and/or toxicological effects (Cascorbi, 2006; Gotanda, Tokumoto, & Hirota, 2015; Ieiri, Higuchi, & Sugiyama, 2009; Maeda & Sugiyama, 2008). Genetic polymorphisms influence the gene expression. SNPs in pharmacokinetic-related genes play an important role in interindividual variations in drug responses (Gotanda et al., 2015; Ieiri et al., 2009; Ma & Lu, 2011; Maeda & Sugiyama, 2008). Genetic variations in the *ABCC3* gene showed large interindividual variability in expression levels (Takechi et al., 2018).

In our study, the genotype/allele distribution of *ABCC3*-1767G/A polymorphism was comparable with study carried out in the population of Caucasians and Japanese by Sasaki et al. (2011) and Fukuda et al. (2010) and dissimilar with study done in the population of African Americans and Japanese by Sasaki et al. (2011). In this study, *ABCC3*-1767G/A polymorphism was neither associated with acquisition of ARV-associated hepatotoxicity nor its severity. However, a higher prevalence of 1767AA genotype was found in HIV patients compared with healthy controls (2.3% vs. 1.3%, OR = 1.71). Similar results were obtained by Fukuda et al. (2010). *ABCC3*-1767G/A polymorphism was not associated with patients with hepatocellular carcinoma (OR = 0.85, 95% CI: 0.42–1.74) (Fukuda & Kawahara, 2010).

The present study was undertaken as a case–control, and the current CD4 count was taken as a substitute for current HIV disease stage. Since the time points for HIV infection are not known, we assumed that the results may be confounded by the duration of HIV infection. In our study, *ABCC3*-1767AA genotype was found to be higher in individuals with early and advanced HIV disease stages in comparison with healthy controls.
TABLE 8  Frequency distribution of ABCC3-1767G/A genotypes in alcohol and NNRTI regimen using HIV patients with hepatotoxicity and without hepatotoxicity

| HIV patients with hepatotoxicity | Alcohol + Nevirapine user | Alcohol nonuser + Nevirapine users | p-Value | OR (95% CI) |
|-------------------------------|---------------------------|-----------------------------------|---------|-------------|
| Genotype ABCC3-1767G/A | N = 5 (%) | N = 18 (%) | | |
| GG | 1 (50.0%) | 4 (44.44%) | — | 1 (Reference) |
| GA | 1 (50.0%) | 4 (44.44%) | .42 | 1.00 (—) |
| AA | 0 (0.0%) | 1 (11.12%) | NS | — |

| HIV patients with hepatotoxicity | Alcohol + Efavirenz user | Alcohol nonuser + Efavirenz users | p-Value | OR (95% CI) |
|-------------------------------|---------------------------|-----------------------------------|---------|-------------|
| Genotype ABCC3-1767G/A | N = 2 | N = 9 | | |
| GG | 0 (0.0%) | 1 (11.12%) | — | 1 (Reference) |
| GA | 2 (100%) | 4 (44.44%) | NC | — |
| AA | 0 (0.0%) | 4 (44.44%) | NS | — |

| HIV patients without hepatotoxicity | Alcohol + Nevirapine user | Alcohol nonUser + Nevirapine users | p-Value | OR (95% CI) |
|-------------------------------|---------------------------|-----------------------------------|---------|-------------|
| Genotype ABCC3-1767G/A | N = 38 (%) | N = 81 (%) | | |
| GG | 18 (47.37%) | 34 (41.98%) | — | 1 (Reference) |
| GA | 17 (44.74%) | 35 (43.20%) | 1.00 | 0.92 (0.38–2.24) |
| AA | 3 (7.89%) | 12 (14.82%) | .44 | 0.47 (0.09–2.16) |

| HIV patients without hepatotoxicity | Alcohol + Efavirenz users | Alcohol nonUser + Efavirenz users | p-Value | OR (95% CI) |
|-------------------------------|---------------------------|-----------------------------------|---------|-------------|
| Genotype ABCC3-1767G/A | N = 6 | N = 6 | | |
| GG | 0 (0.0%) | 0 | — | — |
| GA | 5 (83.33%) | 1 (16.67%) | — | 1 (Reference) |
| AA | 1 (16.67%) | 5 (83.33%) | .08 | 0.04 (0.0–1.29) |

Note: (%), frequency of subjects, odds ratios and 95% CIs were derived from logistic regression models comparing the homozygous wild-type genotype (GG genotype and G allele for ABCC3-1767G/A) with other genotypes. HIV, human immunodeficiency virus; N, number of subjects; NC, not calculable; NNRTI, non-nucleoside reverse transcriptase inhibitors; NS, not significant.

TABLE 9  Multivariate analysis between HIV patients with and without hepatotoxicity

| Variables | B | S.E. | df | p-Value | OR (95% CI) |
|-----------|---|------|----|---------|-------------|
| 1767GG    | 2 | .99  |    |         |             |
| 1767GA    | -.016 | 0.481 | 1 | .97 | 0.98 (0.38–2.52) |
| 1767AA    | 20.48 | 21728.589 | 1 | .99 | 79056108 (0.0— |
| Age       | -.040 | 0.031 | 1 | .20 | 0.96 (0.90–1.02) |
| Sex       | 0.374 | 0.464 | 1 | .42 | 1.45 (0.45–4.63) |
| Tobacco user | 0.370 | 0.594 | 1 | .53 | 1.44 (0.370–4.003) |
| Alcohol user | 0.377 | 0.611 | 1 | .53 | 1.45 (0.44–4.83) |
| NNRTI drug user | 1.518 | 0.534 | 1 | .004 | 4.56 (1.60–12.99) |
| HIV stages (intermediate) | -1.256 | 0.866 | 1 | .14 | 0.28 (0.05–1.55) |
| HIV stages (advanced) | -.0720 | 0.843 | 1 | .39 | 0.48 (0.09–2.54) |

Note: ABCC3-1767G/A polymorphism, age 18–50 years, sex, tobacco user, alcohol user, NNRTI drug user, baseline CD4. Significant values (<0.05) represented in bold.

Abbreviations: HIV, human immunodeficiency virus; NNRTI, non-nucleoside reverse transcriptase inhibitors.
(5.3% vs. 1.3%, OR = 4.73, p = .70; 8.9% vs. 1.3%, OR = 1.89, p = .91). This suggests that the presence of −1767AA genotype may facilitate the risk of advancement of HIV disease.

The gene–environment interactions determine the pathophysiology of the disease (Deng, Newman, & Dunne, 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 and environment. Here, we have chosen the case-only analysis. The consumption of heavy alcohol had a negative impact on the CD4 cell counts of HIV patient’s naïve to ART (Samet, Cheng, & Libman, 2007). In our study, in HIV patients without hepatotoxicity, the occurrence of ABCC3-1767AA genotype was higher in alcohol users as compared to non-users (4.7% vs. 1.1%, OR = 4.28). In HIV patients with and without hepatotoxicity, ABCC3-1767GA genotype showed a risk for acquisition of hepatotoxicity and its severity in nevirapine users (29.4% vs. 16.7%, OR = 1.69; 30.4% vs. 9.1%, OR = 3.34). It supports the idea that individuals with −1767GA genotype and alcohol and nevirapine usage are more prone to develop alcohol- and drug-related hepatotoxicity.

Also, in HIV patients without hepatotoxicity and either taking alcohol + nevirapine or alcohol + efavirenz, the occurrences of ABCC3-1767AA genotype were reduced as compared to alcohol nonusers (7.89% vs. 14.82%; 16.67% vs. 83.33%, respectively).

While comparing HIV patients with and without hepatotoxicity, on multivariate logistic regression analysis, we found that nevirapine is as an independent risk factor for acquisition of hepatotoxicity severity (OR = 4.56, p = .004).

The present study has certain limitations, which are enlisted as follows: (a) It can only ascertain the association, (b) could not define the causality, (c) we had not determined the plasma efavirenz and nevirapine drug levels in our subjects, and (d) we had assigned a ratio of 1:4 for case controls. Although we could not reach up to predetermined adequate numbers in controls, our case–control ratio is about 1:3, which is also a robust ratio.

5 | CONCLUSION

ABCC3-1767GA polymorphism was not significantly correlated with hepatotoxicity status among the HIV patients. Though −1767AA genotype showed a risk for acquisition of hepatotoxicity and advancement of HIV disease, −1767GA genotype may also increase the risk for acquisition of hepatotoxicity and its severity among the HIV patients taking nevirapine ART drug. Transporter genes play an important role in the disposition of drugs since interindividual variabilities in the expression of transporter genes may lead to inappropriate plasma drug concentrations, thereby causing drug toxicity in the liver. Polymorphisms in drug transporter genes play an important role in interindividual variations in drug responses. Hence, further study of ABCC3-1767GA polymorphism should be done in larger sample size in genetically diverse populations and the subsequent correlation of the results with plasma levels of drugs might be helpful to predict the drug-specific response and drug efficiency.

ACKNOWLEDGMENT

We gratefully acknowledge the help of all NARI-Clinical staff and community staff for the recruitment of subjects. We are thankful to Dr. Manisha Ghatre and Dr. Raman Gangakhedkar, clinch incharges for recruitment of subjects from Model Colony clinic and Gadikhana clinic, respectively. The study was supported by a research grant provided by NARI-ICMR India.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

HariOm Singh: Over all supervision. Sonam Lata: Laboratory work. Ranjana Choudhari: Native English, medical and critical review of the manuscript.

ORCID

HariOm Singh https://orcid.org/0000-0002-7251-3097
Ranjana Choudhari https://orcid.org/0000-0002-7474-5983

REFERENCES

Bandara, L. R., & Kennedy, S. (2002). Toxicoproteomics– A new preclinical tool. Drug Discovery Today, 7, 411–418. https://doi.org/10.1016/S1359-6446(02)02211-0
Borst, P., Evers, R., Kool, M., & Wijnholds, J. (2000). A family of drug transporters: The multidrug resistance-associated proteins. Journal of the National Cancer Institute, 92, 1295–1302. https://doi.org/10.1093/jnci/92.16.1295
Busti, A. J., Hall, R. G., & Margolis, D. M. (2004). Atazanavir for the treatment of human immunodeficiency virus infection. Pharmacotherapy, 24, 1732–1747. https://doi.org/10.1592/phco.24.17.1732.52347
Cascorbi, I. (2006). Role of pharmacogenetics of ATP-binding cassette transporters in the pharmacokinetics of drugs. Pharmacology & Therapeutics, 112, 457–473. https://doi.org/10.1016/j.pharmacolther.2006.04.009
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, 326–331. https://doi.org/10.1016/j.neulet.2004.05.061
Fukuda, M., Kawahara, Y., Hirota, T., Akizuki, S., Shigeto, S., Nakajima, H., … Ohnishi, A. (2010). Genetic polymorphisms of hepatic ABC-transporter in patients with hepatocellular carcinoma. Journal of Cancer Therapy, 1, 114–123. https://doi.org/10.4236/jct.2010.13019
Gotanda, K., Tokumoto, T., Hirota, T., Fukae, M., & Ieiri, I. (2015). Sulfasalazine disposition in a subject with 376C>T (nonsense mutation) and 421C>A variants in the ABCG2 gene. *British Journal of Clinical Pharmacology*, 80, 1236–1237.

Greenland, S. (1980). The effect of misclassification in the presence of covariates. *American Journal of Epidemiology*, 112, 564–569. https://doi.org/10.1093/oxfordjournals.aje.a113025

Hitzl, M., Klein, K., Zanger, U. M. et al. (2003). Influence of omeprazole on multi drug resistance protein3 expression in human liver. *Journal of Pharmacokinetics and Experimental Therapeutics*, 304, 524–530. https://doi.org/10.1124/jpet.102.043547

Ieiri, I., Higuchi, S., & Sugiyama, Y. (2009). Genetic polymorphisms of uptake (OATP1B1, 1B3) and efflux (MRP2, BCRP) transporters: Implications for inter-individual differences in the pharmacokinetics and pharmacodynamics of statins and other clinically relevant drugs. *Expert Opinion on Drug Metabolism & Toxicology*, 5, 703–729. https://doi.org/10.1517/17425250902976854

Kool, M., de Haas, M., Scheffer, G. L., Scheper, R. J., van Eijk, M. J., Juijn, J. A., ... Borst, P. (1997). Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. *Cancer Research*, 57, 3537–3547.

Ma, Q., & Lu, A. Y. (2011). Pharmacogenetics, pharmacogenomics, and individualized medicine. *Pharmacological Reviews*, 63, 437–459. https://doi.org/10.1124/pr.110.003533

Maeda, K., & Sugiyama, Y. (2008). Impact of genetic polymorphisms of transporters on the pharmacokinetic, pharmacodynamic and toxicological properties of anionic drugs. *Drug Metabolism and Pharmacokinetics*, 23, 223–235. https://doi.org/10.2133/dmpk.23.223

Mukonzo, J. K., Nanzigu, S., Rekić, D., Waako, P., Röshammar, D., & Ashton, M., ... Aklillu, E. (2011). HIV/AIDS patients display lower relative bioavailability of efavirenz than healthy subjects. *Clinical Pharmacokinetics*, 50, 531–540. https://doi.org/10.2165/11592660-00000000-00000

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, 245–252. https://doi.org/10.4103/0019-5359.99597

Reißler, R. L. S., Servoss, J., & Robbins, G., et al. (2001) The 1st IAS Conference on HIV Pathogenesis and Treatment. Buenos Aires, Argentina.

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, 194–199. https://doi.org/10.1097/QAI.0b013e318142aabb

Sasaki, T., Hirota, T., Ryokai, Y., Kobayashi, D., Kimura, M., Irie, S., ... Ieiri, I. (2011). Systematic screening of human ABCC3 polymorphisms and their effects on MRP3 expression and function. *Drug Metabolism and Pharmacokinetics*, 26, 374–386. https://doi.org/10.2133/dmpk.dmpk-10-rg-103

Takechi, T., Hirota, T., Sakai, T., Maeda, N., Kobayashi, D., & Ieiri, I. (2018). Interindividual differences in the expression of ATP-binding cassette and solute carrier family transporters in human skin: DNA Methylation regulates transcriptional activity of the human ABCC3 gene. *Drug Metabolism and Disposition*, 46, 628–635. https://doi.org/10.1124/117.079061

Uchiumi, T., Hinoshita, E., Haga, S., Nakamura, T., Tanaka, T., Toh, S., ... Kusano, M. (1998). Isolation of a novel human canalicular multispecific organic anion transporter, cMOAT2/MRP3, and its expression in cisplatin-resistant cancer cells with decreased ATP-dependent drug transport. *Biochemical and Biophysical Research Communications*, 252, 103–110. https://doi.org/10.1006.bbrc.1998.9546

Van Dyke, R. B., Wang, L., & Williams, P. L. (2008). Toxicities associated with dual nucleoside reverse-transcriptase inhibitor regimens in HIV-infected children. *The Journal of Infectious Diseases*, 198, 1599–1608. https://doi.org/10.1086/593022

Van Leth, F., Phanuphak, P., Ruxrungtham, K., Baraldi, E., Miller, S., Gazzard, B., ... Lange, J. (2004). Comparison of first-line antiretroviral therapy with regimens including nevirapine, efavirenz, or both drugs, plus stavudine and lamivudine: A randomised open-label trial, the 2NN Study. *Lancet*, 363, 1253–1263. https://doi.org/10.1016/S0140-6736(04)15997-7

Zhou, S. F., Di, Y. M., Chan, E., Du, Y. M., Chow, V. D. W., Xue, C. C., ... Duan, W. (2008). Clinical pharmacogenetics and potential application in personalized medicine. *Current Drug Metabolism*, 9, 738–784.

**How to cite this article:** Singh H, Lata S, Choudhari R, Dhole TN. Prevalence of ABCC3-1767G/A polymorphism among patients with antiretroviral-associated hepatotoxicity. *Mol Genet Genomic Med*. 2020;8:e1124. https://doi.org/10.1002/mgg3.1124