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ORIGINAL ARTICLE

ABCG2 polymorphisms and susceptibility to ARV-associated hepatotoxicity

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Abstract

Background: The *ABCG2* 421C/A polymorphism contributes significantly to the distribution and absorption of antiretroviral (ARV) regimens and is associated with the undesirable side effects of efavirenz.

Methods: To investigate this, we examined *ABCG2* 34G/A (rs2231137) and 421C/A (rs2231142) genetic variations in 149 HIV-infected patients (116 without hepatotoxicity, 33 with ARV-induced hepatotoxicity) and 151 healthy controls through the PCR-restriction fragment length polymorphism (PCR-RFLP) technique.

Results and Discussion: The *ABCG2* 34GA genotype and 34A allele indicated a risk for antiretroviral therapy-associated hepatotoxicity development ($p = 0.09$, OR = 1.58, 95% CI: 0.93–2.69; $p = 0.06$, OR = 1.50, 95% CI: 0.98–2.30). The haplotype GA was associated with hepatotoxicity ($p = 0.042$, OR = 2.37, 95% CI: 1.04–5.43; $p = 0.042$, OR = 2.49, 95% CI: 1.04–5.96). Moreover, when comparing HIV patients with hepatotoxicity to healthy controls, the haplotype GA had an association with an elevated risk for the development of hepatotoxicity ($p = 0.041$, OR = 1.73, 95% CI: 1.02–2.93). Additionally, the association of the *ABCG2* 34GA genotype with the progression of HIV ($p = 0.02$, OR = 1.97, 95% CI: 1.07–3.63) indicated a risk for advanced HIV infection. Furthermore, the *ABCG2* 421AA genotype was linked to tobacco users and featured as a risk factor for the progression of HIV disease ($p = 0.03$, OR = 11.07, 95% CI: 1.09–270.89).

Conclusion: The haplotype GA may enhance the risk of hepatotoxicity development and its severity. Individuals with the *ABCG2* 34A allele may also be at risk for the development of hepatotoxicity. Additionally, individuals with an advanced stage of HIV and the *ABCG2* 34GA genotype may be at risk for disease progression.

KEYWORDS

ABCG2 polymorphism, ARV-associated hepatotoxicity, genetic susceptibility, haplotypes, HIV patients

1 | INTRODUCTION

Antiretroviral therapy (ART) forms the backbone of HIV treatment and management. The primary components of the ART regimen comprise two nucleoside reverse transcriptase inhibitors (NRTIs) in addition to a third effective drug, selected from three distinct drug categories: a non-nucleoside reverse transcriptase inhibitor (NNRTI), an integrase strand transfer inhibitor (INSTI), or a protease inhibitor (PI) combined with a pharmacologic enhancer. However, the selection of an ART regimen is the major challenge in the management of HIV infection due to its efficacy and associated toxicity (Ataro et al., 2019). As a primary organ of drug detoxification and metabolism, the liver is an inherent target for drug toxicity (Kulsharova & Kurmangaliyeva, 2021). NNRTIs are utilized as the main constituent of ARV regimens for managing HIV infections in both children and adults (Heil et al., 2012). Efavirenz (EFV) is the most widely recommended antiretroviral for the treatment of HIV infection. It is an essential part of the first-line regimen prescribed for antiretroviral-naïve HIV-infected patients and is recommended along with two NRTIs. NNRTI (nevirapine and efavirenz) regimens are associated with liver toxicity. However, it is essential to be cautious about the potential adverse drug reactions (ADR) associated with high levels of antiretrovirals (ARVs), such as liver damage in HIV patients.

In HIV patients, ADR may cause hepatotoxicity, affecting disease management (Van Dyke et al., 2008). Efavirenz with emtricitabine induces hepatotoxicity by preventing the transfer of bile acids, characterized by an elevated level of liver aminotransferases (Patil et al., 2015).

In pregnant women, NNRTI, called nevirapine, is commonly utilized for control of HIV infection and gestational transmission (Ajulo et al., 2015). Nevirapine-induced hepatotoxicity is characterized by raised liver enzymes, biliary obstruction, icterus, hepatocellular injury, hepatitis, and ultimately, liver failure (Bera et al., 2012; Hitti et al., 2004). The incidence of typical liver damage in patients on nevirapine therapy ranges from 10% to 18% (Sanne et al., 2005). The occurrence of high-grade, critical hepatotoxicity is documented in 10.8% of individuals treated with efavirenz and 8.9% of individuals treated with nevirapine (Reisler et al., 2001; van Leth et al., 2004). However, lower rates of nevirapine hepatotoxicity (3.19%) have also been reported in the literature (Nagpal et al., 2010).

The activity of drug transporters, in particular P-glycoprotein, is crucial for the distribution process of NNRTIs (Marzolini et al., 2004). Central nervous system-related toxicity, hepatotoxicity, and rash were linked to plasma concentrations of efavirenz and nevirapine (Adkins & Noble, 1998; Gonzalez de Requena et al., 2002; Puzantian, 2002). The transporter gene affects the oral absorption and tissue penetration of nevirapine and efavirenz (Harris & Montaner, 2000; Hu et al., 2015; Schäffler et al., 2001) and contributes to the regulation of cell homeostasis (Gwag et al., 2019).

The ABC transporter protein family consists of ABCG2, also known as BCRP, which aids in transporting bile, lipoproteins, drug substrates, and peptides in various parts of the body such as the BBB, liver, and gut (Ankathil et al., 2018; Eyal et al., 2009; Stieger & Gao, 2015). The gene *ABCG2* is in hepatic and renal tissue, along with a few other tissues. Its primary function is to protect the tissue by inhibiting the accumulation of harmful toxins (Robey et al., 2009).

The *ABCG2* gene, which synthesizes 72 KD membrane proteins from encoded 655 amino acid residues, is located on chromosome 4q22 (Eyal et al., 2009; Vasiliou et al., 2009). Numerous allelic variants of the *ABCG2* gene may affect protein expression, ultimately affecting its transporter function (Bäckström et al., 2003; Zamber et al., 2003). Two important functional variations within the *ABCG2* gene are known: *ABCG2* 34G/A, which leads to the substitution of valine for methionine, and *ABCG2* 421C/A, which causes the replacement of glutamate for lysine. These variations have been linked to detrimental consequences related to the transport of multiple drugs through *ABCG2* (Imai et al., 2002; Kim et al., 2007). The polymorphisms of the *ABCG2* gene (34G/A and 421C/A) are highly prevalent among most ethnic groups and are frequently linked to the susceptibility to several disorders (Ieiri, 2012). The genetic variation of the *ABCG2* gene affects the blood drug concentration of ARVs and may influence the course of HIV disease. Drug bioavailability is known to vary among individuals due to *ABCG2* overexpression (Loscocco et al., 2019). *ABCG2* 421C/A variation increases the chance of developing efavirenz-induced adverse effects (Sánchez Martín et al., 2013; Sánchez-Martín et al., 2016).

The role of *ABCG2* 34G/A and 421C/A variations in ARV-induced hepatotoxicity has not been reported to date. We attempted to investigate the role of *ABCG2* 34G/A and 421C/A polymorphisms in the occurrence of ARV-related hepatotoxicity by comparing its occurrence with HIV-infected patients without hepatotoxicity and healthy controls.

2 | MATERIALS AND METHODS

2.1 | Study participants

This is a cross-sectional prospective study carried out on the Western Indian population. The subject participants of the

study were enrolled in institutional clinics located in Pune, India, during the period of June 2014 to October 2017. A total of 149 subjects who underwent the NNRTI regimen (an antiviral medication used to treat HIV infection) were recruited. All subjects were screened using liver function tests (LFT) and classified as cases and controls based on the grade of hepatotoxicity. 116 HIV patients without hepatotoxicity were considered as a control; 33 HIV-infected patients with hepatotoxicity (Grade III/IV) were considered as a case; and 151 age-matched healthy individuals were considered as a healthy control. Patients with tuberculosis, immunological reconstitution syndrome, hepatitis B, hepatitis C, concurrent infections, or who were taking medications (i.e., acetaminophen, aldomet, amiodarone, etc.) for any other hepatotoxicity were excluded from the study. Along with these, the same aged cohort of 151 individuals were recruited (the same family members were excluded), and all of them tested serum negative for HIV-ELISA, TB, and hepatitis B/C. Clinical data were collected through questionnaires, in-person interviews, and a prepared case file. Liver function tests (LFT) were used to assess the condition of the liver. HIV patients with hepatotoxicity were identified as having bilirubin levels higher than 3.22 mg per mL, SGOT levels greater than 93.8 units per mL, SGPT levels exceeding 229.5 units per mL, and alkaline phosphatase levels above 550.8 units per mL (WHO criteria). HIV patients without hepatotoxicity, also known as controls, were individuals with total bilirubin levels of 1.24 mg per mL, SGOT levels of 32 units per mL, SGPT levels of 34 units per mL, and alkaline phosphatase levels of 108 units per mL (WHO criteria).

The fluorescence-activated cell sorting method was applied to approximate the CD4 cell count, and according to their CD4 status, individuals were divided into various subgroups. An advanced stage of HIV infection was defined by CD4 levels below 200 cells per mm^3, an intermediate stage of 201–350 cells per mm^3, and an early stage of >350 cells per mm^3 (NACO guideline). Murex HBsAg kits and HCV-ELISA kits were utilized to perform ELISA for HBsAg and hepatitis C testing, respectively. The given questionnaire also included information on environmental exposures, including smoking and drinking habits. The ethical clearance was received from the Institutional Ethics Committee (IEC), Pune. All recruited study participants provided valid consent, and the research was executed adhering to the necessary regulations.

2.2 | DNA isolation

Two milliliter of whole blood was withdrawn using aseptic precautions and stored at −80°C. The extraction of DNA from leukocyte pellets was accomplished utilizing the Qiagen Kit (Germany).

2.3 | Genotyping

The PCR-RFLP method was applied for the genotyping of *ABCG2* (34G/A and 421C/A) variants. The primer sequences were taken from Wu et al. (2015) as follows for *ABCG2* 34G/A: FP-5′-AAATGTTCATAGCCAGTTTCTTGGA-3′ and RP-5′-ACAGTAATGTCGAAGTTTTTATCGCA-3′ and 421C/A: FP-5′-GTTGTGATGGGCACTCTGATGGT-3′ and RP-5′-CAAGCCACTTTTCTCATTGTT-3′ (Meissner et al., 2006; Wu et al., 2015). The PCR reaction mixture (20 μL) was prepared with 10 pmol of each primer, 50–100 ng of genomic DNA, 10 mM dNTPs, 10X Taq. buffer, and Taq polymerase enzyme – 1.5 U. *ABCG2* 421C/A reaction conditions were: initial denaturation at 94°C for 5 min, 35 cycles; denaturation at 94°C for 30 s; annealing at 58°C for 30 s; extension at 72°C for 1 min; final extension at 72°C for 7 min. The following conditions for *ABCG2* 34G/A were followed: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s; annealing at 59°C for about 45 s; extension at 72°C for 1 min; and final extension at 72°C for 7 min. The restriction enzymes *BseMI* and *Taa1* were utilized for the digestion of the amplified products of the *ABCG2* 34G/A and 421C/A polymorphisms, respectively (Wu et al., 2015). A total of 10 μL of restriction digestion mixture was prepared to set digestion at 55°C and 65°C overnight for *BseMI* and *Taa1*, respectively. PCR product –3 μL, enzymes – 0.2 μL, buffer – 2.5 μL, and H_2O – 4.7 μL were taken.

Genotypes were seen using 15% polyacrylamide gel, and different sizes of the *ABCG2* (421C/A) genotypes were compared with known markers to visually distinguish them: genotypes *ABCG2* (421C/A): 259 bp for CC, 259 bp, 229, and 30 bp for CA, and 229 and 30 bp for AA genotypes (Meissner et al., 2006), Similarly genotypes *ABCG2* 34G/A, which have 291 bp for the GG genotype, 291 bp, 261 bp, and 30 bp for the GA genotype, and 261 bp and 30 bp -AA genotype (Wu et al., 2015). Other laboratory staff re-genotyped 20% of the samples, and no differences in genotyping were found. To evaluate the genotyping variation, sequencing was performed on 10% of the samples.

2.4 | Statistical analysis

The average age was represented using the mean value and SD. Genetic variations in Hardy–Weinberg equilibrium (HWE) among healthy controls were examined by applying the χ^2 goodness-of-fit test. Genotype frequencies were estimated in different groups of HIV patients with hepatotoxicity, HIV without any hepatotoxicity, and healthy controls. The statistical test used for this analysis was χ^2 (Fisher's exact test for cell size less than 5).

Unconditional binary logistic regression was employed to calculate the odds ratio (OR) and 95% confidence interval (CI). SPSS version 17.0 derived all other statistical calculations (Sole et al., 2006). A probability value ($p < 0.05$) determined the significance level for analysis on a two-sided basis. The relative linkage disequilibrium (LD) value (D') between the two loci was determined using the formula $D' = D_{ij}/D_{max}$ (Singh et al., 2021).

3 | RESULTS

The study enrolled 149 patients with HIV infection, out of whom 33 experienced hepatotoxicity induced by the ARV regimen and 116 did not. Age-matched with the cases, 151 healthy controls were also recruited. The average age and standard deviation of the two groups of HIV-infected and healthy controls ranged from 40.82 ± 7.73 years to 39.36 ± 7.27 years to 37.46 ± 9.77 years, respectively. Table 1 presents the personal and treatment attributes of study groups.

3.1 | *ABCG2* 34G/A and *421C*/A polymorphisms in HIV disease

The distribution of *ABCG2* 421CC, 421CA, 34GG, and 34GA genotypes was nearly equal between HIV patients and healthy controls (75.8% vs. 72.2%; 20.8% vs. 26.5%; 54.4% vs. 62.9%; 41.6% vs. 35.1%), respectively. *ABCG2* 421AA and 34AA genotypes were more prevalent in HIV patients than healthy controls and conveyed a risk for the progression of HIV disease, although statistically insignificant (3.4% vs.1.3%, $p = 0.49$, OR = 2.49, 95% CI: 0.40–18.36; 4.0% vs. 2.0%, $p = 0.38$, OR = 2.35, 95% CI: 0.50–12.28). Likewise, the occurrence of the *ABCG2* 421C and 421A alleles was identical to a greater extent in HIV-infected patients and healthy controls (86.24% vs. 85.43%; 13.75% vs. 14.56%; 75.16% vs. 80.46%; and 24.83% vs. 19.53%) (Table 2).

3.2 | *ABCG2* 34G/A and *421C*/A polymorphisms in HIV patients with and without hepatotoxicity

The distribution of the *ABCG2* 421AA genotype and 421A allele was different between HIV patients with and without hepatotoxicity, probably indicating the risk for hepatotoxicity severity (6.1% vs. 2.6%, $p = 0.58$, OR = 2.76, 95% CI: 0.30–22.17; 19.66% vs. 12.06%, $p = 0.16$, OR = 1.79, 95% CI: 0.81–3.89). The occurrence of *ABCG2* 421CC and 421CA genotypes and 421 C alleles in HIV patients with hepatotoxicity was comparable with that in HIV patients without hepatotoxicity (66.7% vs. 78.4%; 27.3% vs. 19%; 80.33% vs. 87.93%), respectively. Patients

TABLE 1 Characteristics of patients with and without ARV-associated hepatotoxicity, and healthy controls.

Criteria	HIV with hepatotoxicity (grade III and IV)	HIV without hepatotoxicity	Healthy controls
Number	$N = 33$	$N = 116$	$N = 151$
Average age ± standard deviation (years ± SD)	40.82 ± 7.73	39.36 ± 7.27	37.46 ± 9.77
Females	16 (48.5%)	39 (33.6%)	38 (25.2%)
Males	17 (51.5%)	77 (66.4%)	113 (74.8%)
NNRTI regimen			
Nevirapine ($N = 127$)	22 (66.7%)	105 (90.5%)	NA
Efavirenz ($N = 22$)	11 (33.3%)	11 (9.5%)	NA
Alcohol habit			
User ($N = 46$)	7 (21.2%)	39 (33.6%)	0
Non user ($N = 103$)	26 (78.8%)	77 (66.4%)	0
Tobacco habit			
User ($N = 45$)	7 (21.2%)	38 (32.8%)	0
Non-user ($N = 104$)	26 (78.8%)	78 (67.2%)	0
CD4+ status			
<200 ($N = 85$)	15 (45.5%)	70 (60.3%)	NA
201–350 ($N = 44$)	16 (48.5%)	28 (24.1%)	NA
>350 ($N = 20$)	2 (6.0%)	18 (15.6%)	NA

Abbreviation: NA, not applicable.

TABLE 2 Occurrence of *ABCG2 421C*/A and 34G/A polymorphism in HIV patients (with and without hepatotoxicity) and healthy controls.

Genotypes *ABCG2* 421C/A	HIV patients *N* = 149 (%)	Healthy controls *N* = 151 (%)	*p*-value	OR (95% CI)
CC	113 (75.8%)	109 (72.2%)	1	Reference
CA	31 (20.8%)	40 (26.5%)	0.35	0.75 (0.42–1.32)
AA	5 (3.4%)	2 (1.3%)	0.49	2.49 (0.40–18.36)
Alleles *ABCG2* 421C/A	**HIV patients *N* = 298 (%)**	**Healthy controls *N* = 302 (%)**	***p*-value**	**OR (95% CI)**
C	257 (86.24%)	258 (85.43%)	1	Reference
A	41 (13.75%)	44 (14.56%)	0.86	0.94 (0.58–1.52)
Genotypes *ABCG2* 34G/A	**HIV patients *N* = 149 (%)**	**Healthy controls *N* = 151 (%)**	***p*-value**	**OR (95% CI)**
GG	81 (54.4%)	95 (62.9%)	1	Reference
GA	62 (41.6%)	53 (35.1%)	0.23	1.37 (0.83–2.26)
AA	6 (4.0%)	3 (2.0%)	0.38	2.35 (0.50–12.28)
Alleles *ABCG2* 34G/A	**HIV patients *N* = 298 (%)**	**Healthy controls *N* = 302 (%)**	***p*-value**	**OR (95% CI)**
G	224 (75.16%)	243 (80.46%)	1	Reference
A	74 (24.83%)	59 (19.53%)	0.14	1.36 (0.91–2.04)

Note: Presence of CC for CA, AA genotypes and C for A allele, GG for GA and AA genotypes and G for T allele were taken as reference group for statistical analysis.

Abbreviations: *N*, number of subjects; (%), frequency of genotypes/allele.

with and without hepatotoxicity have shown a comparable frequency of the *ABCG2* 34GG, 34GA, and 34AA genotypes as well as the 34G and 34A alleles (66.7% vs. 53.4%; 30.3% vs. 30.3%; 3.0% vs. 3.0%; 81.81% vs. 74.56%; 18.18% vs. 24.43%) (Table 3).

3.3 | *ABCG2* 34G/A and 421C/A polymorphisms and HIV patients hepatotoxicity and healthy controls

Among healthy controls, *ABCG2* 421C/A and 34G/A polymorphisms followed the principle of the HWE ($p = 0.43$, 0.15). The distribution of the *ABCG2* 421AA genotype was different between HIV patients having hepatotoxicity and healthy controls, which might be a genetic risk marker for hepatotoxicity, although it couldn't achieve the significance in our study (6.1% vs. 1.3%; $p = 0.29$, OR = 4.95, 95% CI: 0.47–52.91). The distribution of *ABCG2* 421CC, 421CA genotypes and 421C, 421A alleles was comparable in both HIV patients with hepatotoxicity and healthy control groups (66.7% vs. 72.2%; 27.3% vs. 26.5%; 26.5% vs. 85.43%; 19.66% vs. 14.56%), respectively. The occurrence of the *ABCG2* 34GG, 34GA, and 34AA genotypes, as well as the 34G and 34A alleles, were nearly the same in both groups (66.7% vs. 62.9%; 30.3% vs. 35.1%; 3.0% vs. 3.0%; 81.81% vs. 80.46%; and 18.18% vs. 19.53%).

The frequency of *ABCG2* 421AA genotype in HIV patients without hepatotoxicity was dissimilar from healthy controls, indicating susceptibility for hepatotoxicity (2.6% vs. 1.3%; $p = 0.85$, OR = 1.80, 95% CI: 0.24–15.74). In both groups, the occurrence of the *ABCG2* 421CC, 421CA genotypes, as well as the 421C and 421A alleles, was almost alike (19% vs. 26.5%; 2.6% vs. 1.3%; 87.93% vs. 85.43%; 12.06% vs. 14.56%). The prevalence of the *ABCG2* 34A allele was different between patients without hepatotoxicity and healthy controls, which revealed an insignificant risk of acquiring hepatotoxicity (62.72% vs. 19.53%, $p = 0.06$, OR:1.50, 95% CI: 0.98–2.30). The distribution of *ABCG2* 34GA and 34AA genotypes didn't vary significantly between groups without hepatotoxicity and healthy controls and indicated an elevated risk, not reaching statistical significance for the acquisition of hepatotoxicity (44.8% vs. 35.1%; $p = 0.09$, OR = 1.58, 95% CI: 0.93–2.69; 4.3% vs. 2.0%, $p = 0.32$, OR = 2.66, 95% CI: 0.53–14.65) (Table 4).

3.4 | Haplotypes analysis

Table 5 depicts the haplotype distribution of *ABCG2* (*421C*/A and 34G/A) gene variations in HIV patients and

TABLE 3 Occurrence of *ABCG2* 421C/A and 34G/A polymorphism in patients with and without ARV-associated hepatotoxicity.

Genotypes *ABCG2* 421C/A	Patients with hepatotoxicity *N* = 33 (%)	Patients without hepatotoxicity *N* = 116 (%)	*p*-value	OR (95% CI)
CC	22 (66.7%)	91 (78.4%)	1	Reference
CA	9 (27.3%)	22 (19%)	0.36	1.69 (0.62–4.55)
AA	2 (6.1%)	3 (2.6%)	0.58	2.76 (0.30–22.17)
Alleles *ABCG2* 421C/A	**Patients with hepatotoxicity *N* = 66(%)**	**Patients without hepatotoxicity *N* = 232(%)**	***p*-value**	**OR (95% CI)**
C	53 (80.33%)	204 (87.93%)	1	Reference
A	13 (19.66%)	28 (12.06%)	0.16	1.79 (0.81–3.89)
Genotypes *ABCG2* 34G/A	**Patients with hepatotoxicity *N* = 33 (%)**	**Patients without hepatotoxicity *N* = 116 (%)**	***p*-value**	**OR (95% CI)**
GG	22 (66.7%)	62 (53.4%)	1	Reference
GA	10 (30.3%)	49 (42.2%)	0.27	0.58 (0.23–1.42)
AA	1 (3.0%)	5 (4.3%)	0.97	0.56 (0.02–5.50)
Alleles *ABCG2* 34G/A	**Patients with hepatotoxicity *N* = 66 (%)**	**Patients without hepatotoxicity *N* = 232 (%)**	***p*-value**	**OR (95% CI)**
G	54 (81.81%)	173 (74.56%)	1	Reference
A	12 (18.18%)	59 (24.43%)	0.29	0.65 (0.31–1.36)

Note: Presence of CC for CA, AA genotypes and C for A allele, GG for GA and AA genotypes and G for T allele were taken as reference group for statistical analysis.

Abbreviations: *N*, number of subjects; (%), frequency of genotypes/allele.

a healthy control group. Linkage disequilibrium (LD) D′ between the sites for any of the three groups was unable to reach any significance level (D′ = 0.467, 0.386, and 0.386). Although significant differences were not observed in LD values (Dij) between any two study groups, it is anticipated that individual gene variations, collectively, might have a synergistic effect on the susceptibility to the development of hepatotoxicity.

The prevalence of haplotype AG (*ABCG2 421**G/34*A) was significantly higher in patients with hepatotoxicity than without hepatotoxicity and healthy control groups; it was linked with an elevated risk of hepatotoxicity (0.197 vs. 0.087, $p = 0.042$, OR = 2.37, 95% CI: 1.04–5.43; 0.197 vs. 0.120; $p = 0.042$, OR = 2.49, 95% CI: 1.04–5.96). The haplotypes CG (*ABCG2 421**C/34*G), CA (*ABCG2 421**C/34*A), and AA (*ABCG2 421**A/34*A) were similarly distributed between patients with hepatotoxicity and healthy controls (0.621 vs. 0.683; 0.181 vs. 0.170; 0.024 vs. 0.024). The frequency of haplotypes CG (*ABCG2 421**C/34*G) and CA (*ABCG2 421**C/34*A) was comparable in patients with and without hepatotoxicity (0.621 vs. 0.647; 0.181 vs. 0.235).

The occurrence of haplotype CA (*ABCG2 421**C/34*A) was significantly different between patients with HIV without hepatotoxicity and healthy controls, and the association denotes the risk for the development of hepatotoxicity (0.235 vs. 0.170; $p = 0.041$, OR = 1.73, 95% CI: 1.02–2.93). Patients without hepatotoxicity and healthy controls had almost similar distributions of the haplotypes CG (*ABCG2 421**C/34*G), AG (*ABCG2 421**A/34*G), and AA (*ABCG2 421**A/34*A) (0.647 vs. 0.683; 0.087 vs. 0.120; 0.029 vs. 0.024) (Table 5).

3.5 | *ABCG2* 34G/A and *421C*/A polymorphisms in stages of HIV disease

The prevalence of *ABCG2 421CC*, *421CA*, and *421AA* genotypes was observed to be comparable between the early stage of HIV and the healthy group (60.0% vs. 72.2%; 35.0% vs. 26.5%; 5.0% vs. 1.3%); the intermediate stage of HIV disease and healthy controls (72.7% vs. 72.2%; 20.5% vs. 26.5%; 6.8% vs. 1.3%); and the advanced stage of HIV disease and the healthy group (81.2% vs. 72.2%; 17.6% vs. 26%). The prevalence of the *ABCG2* 34GA genotype significantly differed in the advanced stage of HIV disease from healthy controls and was reflected as a higher risk of HIV disease advancement (40.0% vs. 26.5%, $p = 0.02$, OR = 1.97, 95% CI: 1.07–3.63).

The distribution of the *ABCG2* 34AA genotype in the advanced stage of HIV disease against a healthy group (4.7% vs. 1.3%, $p = 0.14$, OR: 4.64), the intermediate HIV disease stage against a healthy group (2.3% vs. 1.3%, $p = 0.89$, OR = 2.10), and the early stage of HIV disease against a healthy group(5.0% vs. 1.3%, $p = 0.54$, OR = 6.81) manifested risk, although it was insignificant for the

TABLE 4 Occurrence of *ABCG2 421C*/A and 34G/A polymorphism in patients with and without ARV-associated hepatotoxicity and healthy controls.

Genotypes *ABCG2* 421C/A	Patients with hepatotoxicity *N* = 33 (%)	Healthy controls *N* = 151 (%)	*p*-value	OR (95% CI)
CC	22 (66.7%)	109 (72.2%)	1	Reference
CA	9 (27.3%)	40 (26.5%)	0.97	1.11 (0.43–2.82)
AA	2 (6.1%)	2 (1.3%)	0.29	4.95 (0.47–52.91)
Allele *ABCG2* 421C/A	**Patients with hepatotoxicity *N* = 66 (%)**	**Healthy controls *N* = 302 (%)**	***p*-value**	**OR (95% CI)**
C	53 (80.33%)	258 (85.43%)	1	Reference
A	13 (19.66%)	44 (14.56%)	0.39	1.44 (0.68–2.99)
Genotypes *ABCG2* 34G/A	**Patients with hepatotoxicity *N* = 33 (%)**	**Healthy controls *N* = 151 (%)**	***p*-value**	**OR (95% CI)**
GG	22 (66.7%)	95 (62.9%)	1	Reference
GA	10 (30.3%)	53 (35.1%)	0.77	0.81 (0.33–1.97)
AA	1 (3.0%)	3 (2.0%)	0.73	1.44 (CI limit invalid)
Alleles *ABCG2* 34G/A	**Patients with hepatotoxicity *N* = 66(%)**	**Healthy controls *N* = 302 (%)**	***p*-value**	**OR (95% CI)**
G	54 (81.81%)	243 (80.46%)	1	Reference
A	12 (18.18%)	59 (19.53%)	0.93	0.92 (0.43–1.90)
Genotypes *ABCG2* 421C/A	**Patients without hepatotoxicity *N* = 116(%)**	**Healthy controls *N* = 151 (%)**	***p*-value**	**OR (95% CI)**
CC	91 (78.4%)	109 (72.2%)	1	Reference
CA	22 (19%)	40 (26.5%)	0.21	0.66 (0.35–1.24)
AA	3 (2.6%)	2 (1.3%)	0.85	1.80 (0.24–15.74)
Allele *ABCG2* 421C/A	**Patients without hepatotoxicity *N* = 232 (%)**	**Healthy controls *N* = 302 (%)**	***p*-value**	**OR (95% CI)**
C	204 (87.93%)	258 (85.43%)	1	Reference
A	28 (12.06%)	44 (14.56%)	0.47	0.80 (0.47–1.38)
Genotypes *ABCG2* 34G/A	**Patients without hepatotoxicity *N* = 116(%)**	**Healthy controls *N* = 151 (%)**	***p*-value**	**OR (95% CI)**
GG	59 (50.9%)	95 (62.9%)	1	Reference
GA	52 (44.8%)	53 (35.1%)	0.09	1.58 (0.93–2.69)
AA	5 (4.3%)	3 (2.0%)	0.32	2.66 (0.53–14.65)
Alleles *ABCG2* 34G/A	**Patients without hepatotoxicity *N* = 232 (%)**	**Healthy controls *N* = 302 (%)**	***p*-value**	**OR (95% CI)**
G	170 (73.27%)	243 (80.46%)	1	Reference
A	62 (62.72%)	59 (19.53%)	**0.06**	**1.50 (0.98–2.30)**

Note: For statistical inference, presence of CC for CA, AA genotypes and C for A allele, GG for GA and AA genotypes and G for T allele were treated as reference group. $p < 0.05$ in bold represents significance level.

Abbreviations: *N*, number of subjects; (%), frequency of genotypes/allele.

progression of HIV infection. An early stage of HIV disease (40.0% vs. 72.2%) as well as an advanced stage of HIV disease (55.3% vs. 72.2%) both revealed a lesser prevalence of the *ABCG2* 34GG genotype, whereas in an intermediate stage of HIV disease, the prevalence was almost identical with that of healthy controls (72.7% vs. 72.2%) (Table 6).

3.6 | *ABCG2* 34G/A and *421C*/A polymorphisms and HIV patients using tobacco and alcohol

In HIV patients, the presence of *the ABCG2* 421AA genotype was significantly greater in smokers compared with

TABLE 5 Occurrence of haplotypes of *ABCG2 421C*/A and 34G/A polymorphisms between HIV patients with and without ARV-associated hepatotoxicity and healthy controls.

Haplotypes *ABCG2* 421C/A, 34G/A	Patients with hepatotoxicity *N* = 66(%)	Patients without hepatotoxicity *N* = 232 (%)	*p*-value	OR (95% CI)
CG	0.621	0.647	1	Reference
CA	0.181	0.235	0.36	0.68 (0.30–1.53)
AG	0.197	0.087	**0.042**	**2.37 (1.04–5.43)**
AA	0.000	0.029	–	–
Haplotypes *ABCG2* 421C/A, 34G/A	**Patients with hepatotoxicity *N* = 66 (%)**	**Healthy controls *N* = 302 (%)**	***p*-value**	**OR (95% CI)**
CG	0.621	0.683	1	Reference
CA	0.181	0.170	0.55	1.30 (0.55–3.12)
AG	0.197	0.120	**0.042**	**2.49 (1.04–5.96)**
AA	0.024	0.024	0.80	0.07 (0.00–42.75)
Haplotypes *ABCG2* 421C/A, 34G/A	**Patients without hepatotoxicity *N* = 232(%)**	**Healthy controls *N* = 302(%)**	***p*-value**	**OR (95% CI)**
CG	0.647	0.683	1	Reference
CA	0.235	0.170	**0.041**	**1.73 (1.02–2.93)**
AG	0.087	0.120	0.98	1.01 (0.48–2.11)
AA	0.029	0.024	0.23	2.31 (0.59–9.02)

Note: (%) = frequency of subjects, Odds ratios, and 95% CIs were derived from logistic regression models comparing the haplotype GG with other haplotypes. $p < 0.05$ in bold represents significance level.

TABLE 6 HIV stage-wise and study group-wise distribution of *ABCG2 421C*/A and 34G/A polymorphisms.

Genotypes *ABCG2* 421C/A	Healthy controls *N* = 151 (%)	Early HIV disease stage *N* = 20 (%)	OR (P)	Intermediate HIV disease stage *N* = 44 (%)	OR (P)	Advanced HIV disease stage *N* = 85 (%)	OR (P)
CC	109 (72.2%)	12 (60.0%)	1 (Ref)	32 (72.7%)	1 (Ref)	69 (81.2%)	1 (Ref)
CA	40 (26.5%)	7 (35.0%)	1.59 (0.52)	9 (20.5%)	0.77 (0.66)	15 (17.6%)	0.59 (0.16)
AA	2 (1.3%)	1 (5.0%)	4.54 (0.72)	3 (6.8%)	5.11 (0.16)	1 (1.2%)	0.79 (1.00)
Genotypes *ABCG2* 34G/A	**Healthy controls *N* = 151 (%)**	**Early HIV disease stage *N* = 20(%)**	**OR (P)**	**Intermediate HIV disease stage *N* = 44 (%)**	**OR (P)**	**Advanced HIV disease stage *N* = 85 (%)**	**OR (P)**
GG	109 (72.2%)	8 (40.0%)	1 (Ref)	26 (59.1%)	1 (Ref)	47 (55.3%)	1 (Ref)
GA	40 (26.5%)	11 (55.0%)	**3.75 (0.01)**	17 (38.6%)	1.78 (0.15)	34 (40.0%)	**1.97 (0.02)**
AA	2 (1.3%)	1 (5.0%)	6.81 (0.54)	1 (2.3%)	2.10 (0.89)	4 (4.7%)	4.64 (0.14)

Note: CC for CA and AA genotypes and GG for GA and AA genotypes were reference groups for statistical analysis purpose. Significance is taken at $p < 0.05$, which is represented in bold.

Abbreviations: *N*, number of subjects; (%), frequency of genotypes/allele.

non-smokers and found to be linked with an enhanced risk for HIV disease progression (8.9% vs. 1.0%; *p*: 0.03, OR = 11.07; 95% CI: 1.09–270.89). The occurrence of *ABCG2* 421CC and 421CA genotypes was not different between smokers and non-smokers among HIV patients (66.7% vs. 79.8%; 24.4% vs. 19.2%). *ABCG2* 34GG, 34GA, and 34AA genotypes did not vary between smokers and non-smokers (64.4% vs. 50.0%; 33.3% vs. 45.2%; 2.2% vs. 4.8%).

In HIV patients, alcohol users and non-users had a different representation of genotypes of *ABCG2* 421AA, 421CA, and 421CC (80.4% vs. 73.8%, 80.4% vs. 23.3%, and 4.3% vs. 2.9%, respectively). HIV-infected people consuming alcohol had a comparable prevalence of *ABCG2* 34GG, 34GA, and 34AA genotypes (54.3% vs. 54.4%; 41.3% vs. 41.7%; 4.3% vs. 3.9%) as compared to non-alcohol consumers. (Table 7).

TABLE 7 Occurrence of *ABCG2 421C*/A and 34G/A genotypes and addiction habits of HIV patients.

Genotypes *ABCG2* 421C/A	Tobacco user *N* = 45 (%)	Tobacco non-user *N* = 104 (%)	*p*-value	OR (95% CI)
CC	30 (66.7%)	83 (79.8%)	1	Reference
CA	11 (24.4%)	20 (19.2%)	0.45	1.52 (0.60–3.83)
AA	4 (8.9%)	1 (1.0%)	**0.03**	**11.07 (1.09–270.89)**
Genotypes *ABCG2* 34G/A	**Tobacco user *N* = 45 (%)**	**Tobacco non-user *N* = 104 (%)**	***p*-value**	**OR (95% CI)**
GG	29 (64.4%)	52 (50.0%)	1	Reference
GA	15 (33.3%)	47 (45.2%)	0.19	0.57 (0.26–1.27)
AA	1 (2.2%)	5 (4.8%)	0.92	0.63 (0.03–6.42)
Genotypes *ABCG2* 421C/A	**Alcohol user *N* = 46 (%)**	**Alcohol non-user *N* = 103 (%)**	***p*-value**	**OR (95% CI)**
CC	37 (80.4%)	76 (73.8%)	1	Reference
CA	7 (80.4%)	24 (23.3%)	0.38	0.60 (0.21–1.64)
AA	2 (4.3%)	3 (2.9%)	0.88	1.37 (0.15–10.70)
Genotypes *ABCG2* 34G/A	**Alcohol user *N* = 46 (%)**	**Alcohol non-user *N* = 103 (%)**	***p*-value**	**OR (95% CI)**
GG	25 (54.3%)	56 (54.4%)	1	Reference
GA	19 (41.3%)	43 (41.7%)	0.87	0.99 (0.45–2.15)
AA	2 (4.3%)	4 (3.9%)	0.74	1.12 (0.13–7.85)

Note: CC for CA and AA genotypes and GG for GA and AA genotypes were taken as reference group for statistical inference. Level of significance at $p < 0.05$.
Abbreviations: *N*, Number of subjects; (%), frequency of genotypes/allele.

3.7 | *ABCG2* 34G/A and *421C*/A polymorphisms and HIV patients on NNRTI regimens

In HIV patients with hepatotoxicity, *ABCG2* 421CA, 34GA genotypes varied between recipients of efavirenz and nevirapine drugs (18.2% vs. 31.8%; 72.7% vs. 31.8%). The *ABCG2* 34GG genotype was more prevalent in efavirenz users compared to nevirapine users (72.72% vs. 63.6%). HIV patients on efavirenz with the *ABCG2* 421CA genotype had an increased risk of developing ARV-associated hepatotoxicity (45.5% vs. 2.9%, $p = 0.05$, OR = 4.17, 95% CI: 0.96–18.00). Though the *ABCG2* 421CC genotype appeared lesser in efavirenz than nevirapine users (54.5% vs. 81.0%), the *ABCG2* 34GA, 34GG genotypes were observed alike in both groups (45.5% vs. 44.8%; 54.5% vs. 50.5%) (Table 8).

4 | DISCUSSION

Our study is the maiden report, highlighting the association of certain haplotypes *ABCG2* 421C/A and 34G/A polymorphisms with ARV hepatotoxicity. ARV regimens have been linked to hepatotoxicity. The liver is one of many tissues that express *ABCG2*. *ABCG2* is a key player in the disposition of substrates as well as in drug–drug interactions (International Transporter C et al., 2010). Target tissues, such as the brain, placenta, and liver, are protected by *ABCG2* from xenobiotics and toxic metabolites. Genetic variants of *ABCG2* affect the expression and are linked to the disease outcome. Reduced expression of transporter protein is seen in several tissues as a result of the *ABCG2* c.421 C/A polymorphism, which makes ABCG2 protein unstable, especially in the endoplasmic reticulum, thus increasing the vulnerability to denaturation (Furukawa et al., 2009; Kobayashi et al., 2005; Prasad et al., 2013; Tanaka et al., 2015).

Our investigation found that the prevalence of the *ABCG2* 34G/A polymorphism in our healthy control group differed when compared with studies done by Bäckström et al. (2003), Bosch et al. (2005), however, it is comparable with studies conducted by Fischer et al. (2007), Lee, Jeong, et al. (2007), Słomka et al. (2020), Wang et al. (2004), Wang et al. (2007). *ABCG2 421AA* and 34AA genotypes showed an elevated risk for advancement of HIV disease when patients with HIV infection were compared with healthy subjects ($p = 0.49$, OR: 2.49; $p = 0.38$, OR = 2.35). On comparison of non-hepatotoxic HIV patients with a healthy control group, the *ABCG2* 34GA genotype and 34A allele

TABLE 8 Occurrence of *ABCG2 421C*/A and 34G/A polymorphisms in patients on NNRTI regimens.

HIV patients with hepatotoxicity				
Genotypes *ABCG2* 421C/A	**Efavirenz users *N* = 11 (%)**	**Nevirapine users *N* = 22 (%)**	***p*-value**	**OR (95% CI)**
CC	7 (63.6%)	15 (68.2%)	1	Reference
CA	2 (18.2%)	7 (31.8%)	0.92	0.61(0.07–4.77)
AA	2 (18.2%)	0 (0.0%)	–	–
Genotypes *ABCG2* 34G/A	**Efavirenz users *N* = 11 (%)**	**Nevirapine users *N* = 22 (%)**	***p*-value**	**OR (95% CI)**
GG	8 (72.72%)	14 (63.6%)	1	Reference
GA	3 (27.27%)	7 (31.8%)	0.95	0.75 (0.11–4.76)
AA	0 (0.0%)	1 (4.5%)	–	–
HIV patients without hepatotoxicity				
Genotypes *ABCG2 421C/A*	**Efavirenz users *N* = 11 (%)**	**Nevirapine user *N* = 105 (%)**	***p*-value**	**OR (95% CI)**
CC	6 (54.5%)	85(81.0%)	1	Reference
CA	5 (45.5%)	17 (2.9%)	0.05	4.17 (0.96–18.00)
AA	0 (0.0%)	3 (2.9%)	–	–
Genotypes *ABCG2* 34G/A	**Efavirenz users *N* = 11 (%)**	**Nevirapine users *N* = 105 (%)**	***p*-value**	**OR (95% CI)**
GG	6 (54.5%)	53 (50.5%)	1	Reference
GA	5 (45.5%)	47 (44.8%)	0.82	0.94(0.23–3.79)
AA	0 (0.0%)	5 (4.8%)	–	–

Note: CC for CA and AA genotypes and GG for GA and AA genotypes were considered as reference group for statistical analysis.

Abbreviations: *N* = number of subjects; (%), frequency of genotypes/allele.

have not shown significant risk for the development of hepatotoxicity ($p = 0.09$, OR = 1.58; $p = 0.06$, OR = 1.50). Breast cancer risk has been linked to the 34GA and 34AA genotypes of *ABCG2* (Wu et al., 2015). In the Korean population, the *ABCG2* 34G/A polymorphism was often seen (Kim et al., 2010).

The frequency of the *ABCG2* 421C/A polymorphism in our healthy controls was different from studies done by Lee, Babakhanian, et al. (2007), Lee, Jeong, et al. (2007) , Bäckström et al. (2003), Bosch et al. (2005), Fischer et al. (2007), Maekawa et al. (2006), Wang et al. (2004). *ABCG2* 421AA genotype and 421A allele carried a statistically non-significant risk for severe hepatotoxicity ($p = 0.58$, OR = 2.76; $p = 0.16$, OR = 1.79). An elevated risk of non-papillary renal cell cancer was associated with the *ABCG2* 421C/A polymorphism (Korenaga et al., 2005). Patients with prostate cancer with the *ABCG2* 421CA genotype had a higher likelihood of survival in the past 15 months than those with the 421CC genotype (Hahn et al., 2006). An increased risk of efavirenz-induced adverse effects has been linked to the *ABCG2* 421C/A polymorphism (Sánchez Martín et al., 2013; Sánchez-Martín et al., 2016).

To analyze the synergistic effect of SNPs (421C/A and 34G/A) in the *ABCG2* gene cluster, we additionally examined haplotype. The haplotype GA was significantly correlated with a greater incidence of hepatotoxicity ($p = 0.042$, OR = 2.37; $p = 0.042$, OR = 2.49). Since the haplotype GA is significantly linked with the development of hepatotoxicity ($p = 0.041$; OR = 1.73), we state that HIV-infected patients with haplotype GA are susceptible to the development of hepatotoxicity.

HIV disease staging was based on the recent CD4 count. Due to the non-availability of data related to milestones in the timeline of the natural course of HIV infection, our results are subjected to the confounding bias of an unknown time span. Our analysis revealed the significant association of the *ABCG2* 34GA genotype with the elevated risk of advancement of HIV infection ($p = 0.02$, OR = 1.97). Findings unwrap the role of the *ABCG2* 34GA genotype in the progression of HIV disease.

The cause–effect relationship of the disease is determined by gene–environment interactions (Deng et al., 2004; Greenland, 1980). To see the impact of gene–environment interaction, a case-only design compared to a

case–control method is preferred (Chen et al., 2011). To assess the risk of progressive HIV disease in alcohol and cigarette users, a case-only method was adopted in our analysis. Excessive use of alcohol in HIV-infected people reduces the CD4 cell count but not the effectiveness of combinational ART (Samet et al., 2007). In our analysis, the *ABCG2* 421AA genotype in tobacco smokers highlighted a significantly increased risk of HIV disease progression ($p = 0.03$, OR = 11.07) when compared with tobacco non-users. This suggests that HIV patients using tobacco along with the *ABCG2* 421AA genotype may exhibit enhanced susceptibility to HIV disease progression due to the additive effects of both risk factors through gene–environment interactions. This has clinical relevance for public health, as there exists biological plausibility of enhancing control on HIV progression through successful implementation of tobacco cessation programs in HIV patients, especially with the *ABCG2* 421AA genotype (Bhalerao & Cucullo, 2022; Mu et al., 2018; Singh et al., 2018).

HIV patients with the *ABCG2* 421CA genotype and recipients of the efavirenz drug showed a significantly higher risk of hepatotoxicity ($p = 0.05$, OR = 4.17). Male patients with the *ABCG2* 421CC genotype had an increased risk of anti-tuberculosis drug-induced hepatotoxicity (OR = 1.615, $p = 0.011$) (Wang et al., 2022). This suggests that HIV-infected individuals having the *ABCG2* 421CA genotype may enhance the risk of efavirenz-induced hepatotoxicity. The finding has potential implications for the application of personalized medication for the clinical management of HIV patients with the *ABCG2* 421CA genotype.

A few restraints are limiting the study outcomes. Firstly, we could explore only an association between variables rather than causality. The cross-sectional study design lacks the inherent ability to establish a cause–effect relationship. Secondly, the study lacks an estimation of the plasma efavirenz and nevirapine concentrations, limiting the objective assessment of causality. Additionally, despite aiming for a case–control ratio of 1:4, we faced challenges in enrolling matched controls, restricting us to a case–control ratio of approximately 1:3. The causality assessment was determined using M&V scale scoring (vary from 6 to 20), corresponding to the sum of the points attributed to each parameter. We assessed according to the observed score: definite (score > 17), probable (score 14–17), possible (score 10–13), unlikely (score 6–9), and excluded (score < 6). We did not find any patients as possible cases out of 33.

5 | CONCLUSIONS

The present study suggests that the *ABCG2* 34A allele and haplotype GA may contribute to ARV-induced hepatotoxicity. *ABCG2 34*GA genotype may be an independent facilitator of HIV disease progression. The *ABCG2* 421AA genotype, along with tobacco addiction, increases the risk of HIV disease progression. HIV patients having the *ABCG2* 421CA genotype and taking efavirenz are more likely to develop drug-induced hepatotoxicity.

6 | FUTURE DIRECTIONS

ABCG2 expression in the liver controls the transportation of drugs. Genetic variation in *ABCG2* has been associated with the expression and altered transporter function and has negative effects on drug transportation. However, the role of these transporter genes in the variation of plasma ARV drug concentration has not been addressed. Hence, studies on *ABCG2* 421C/A and 34G/A polymorphisms among patients with ARV-associated hepatotoxicity should be carried out in a statistically significant sample size in the same and other genetically diverse populations to validate the findings of the present study and address the role of transporter genes in ARV-associated hepatotoxicity.

Further research should be directed to find the additional significant predictor(s) of ARV-induced hepatotoxicity and its advancement. *ABCG2* 421CA genotype may potentiate the drug-induced hepatotoxicity of the efavirenz drug. Our finding, if substantiated by future prospective studies, will lead to potential clinical implications in the application of personalized medication for the management of HIV patients with the *ABCG2* 421CA genotype. HIV patients with the *ABCG2* 421CA genotype receiving ART should undergo periodical monitoring for early indicators of liver toxicity.

AUTHORS' CONTRIBUTION

HariOm Singh: overall supervision and writing of the manuscript. Kishore Dhotre: Genotyping work. Shyamveer: writing of the manuscript. Ranjana Choudhari: clinical input and native English language. Amita Verma: review of manuscript and concept; Supriya D. Mahajan: concept and review of manuscript; Nemat Ali: critical review and English language.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

INSTITUTIONAL REVIEW BOARD STATEMENT

Ethical approval No.: NARI/EC/ICF version 1.0, dated August 28, 2013.

INFORMED CONSENT STATEMENT/ ETHICAL STATEMENT

Witten Informed consent was obtained from all individual participants included in the study (ICF version 1.0 dated 18 April 2011). The ethical clearance was obtained from the Institutional Ethics Committee (IEC), ICMR-NARI, Pune.

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