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Research Article

Genetic Variation and Drug-Induced Liver Injury in Patients on Simvastatin

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Abstract

Drug-induced liver injury, or DILI, is a primary reason for heart failure. Uridine diphosphate glucuronosyltransferase 1-1 encoded by the UDP glucuronosyltransferase family 1-member A1 gene (*UGT1A1*) helps prevent DILI by taking part in reactions that conjugate glucuronic acid with bilirubin. *UGT1A1* variations are reported to be linked with DILI. This study investigated the association between genetic variations in *UGT1A1* and hepatic adverse effects in patients treated with simvastatin. Our results show that *rs4148325* is not significantly associated with elevated bilirubin levels. Therefore, our study suggests that genotypic variations in the *UGT1A1* gene may be responsible for simvastatin-related increases in bilirubin levels in our patient cohort. We conclude that genotyping patients on simvastatin for variations in the *UGT1A1* gene may not help predict whether these patients are more likely to suffer from liver injury and consequently can change the drug or dose of the drug.

Keywords: Drug-induced liver injury, hepatic adverse effects, simvastatin, pharmacogenomics, *UGT1A1*

Introduction

The most commonly observed factor in post-marketing drug withdrawals due to safety-related issues is drug-induced liver injury or DILI (Onakpoya, Heneghan, and Aronson 2016, Center for drug evaluation and research 2009). In the US, DILI is the primary reason for acute hepatic failure (Hoofnagle and Björnsson 2019, Ostapowicz et al. 2002). Nevertheless, the diagnosis of DILI remains a significant challenge as its clinical presentation is quite similar to other types of hepatic injury. However, only after eliminating other forms of hepatic pathologies, including malignancy and other related factors, can DILI be accurately diagnosed (Hoofnagle and Björnsson 2019, Center for drug evaluation and research 2009). The tests for discerning drug-induced liver problems during clinical trials include total bilirubin, alanine transferase (ALT) serum levels, and aspartate transferase (AST). These tests are also referred to as liver function tests (LFTs). DILI or severe hepatocellular injury is usually indicated by "Hy's Law," in which a combination of total bilirubin elevation more than twice the upper limit of normal (ULN) and LFT elevation is more than thrice the ULN(Center for drug evaluation and research 2009). However, other drugs, such as statins, aspirin, and heparin, cause slight elevations in the aminotransferase enzymes without leading to severe liver damage(Center for drug evaluation and research 2009). On the other hand, genetic predispositions to hyperbilirubinemia have also been reported.

The formation of the water-soluble glucuronide of bilirubin which is then excreted in bile is catalyzed by uridine diphosphate (UDP) glucuronosyltransferase 1-1 encoded by UDP

glucuronosyltransferase family 1 member A1 gene (*UGT1A1*) (Strassburg 2008). *UGT1A1* variations have been reported to be linked with Gilbert's syndrome, characterized by elevated levels of unconjugated bilirubin and jaundice without the presence of aminotransferase elevations. The scientific literature suggests that the *UGT1A1*28* allele, a TA repeat polymorphism found in the promotor region, is responsible for most cases (Bosma et al. 1995). In contrast to the 6 TA repeats, [A(TA)6TAA] found in individuals harboring the *UGT1A1*28* polymorphism and have ~70% diminished expression of the *UGT1A1* gene, the more common allele possesses 7 TA repeats [A(TA)7TAA] (Strassburg 2008). *UGT1AQ*28* homozygotes usually suffer from Gilbert's syndrome, in which a lower *UGT1A1* gene expression and diminished bilirubin metabolism are observed. Other less common genetic variants of *UGT1A1* have also been associated with Gilbert's syndrome (Strassburg 2008, Gammal et al. 2016).

Simvastatin differs from other statins because it is a prodrug, and only after metabolism from an inactive form does the drug performs its lipid-lowering effect. An equilibrium is established between the active and inactive forms of the drug. The UGT superfamily is responsible for catalyzing the activation of simvastatin acid into its lactone form via the formation of acyl glucuronide intermediate (Prueksaritanont et al. 2002). As mentioned earlier, genetic variations have been known to affect UGT activity, and polymorphisms for almost all active *UGT1A* genes have been reported. A short tandem repeat (STR) of the length of 5-8 dinucleotide (TA) in the *UGT1A1* promotor is the most common *UGT1A1* genetic polymorphism (Beutler, Gelbart, and Demina 1998, Williams et al. 2008). Multiple genotypes emerge from this polymorphism, some of which inhibit promotor activity and decrease protein expression, especially with increased TA repeats in the polymorphism. This genetic variation bears great significance in clinical practice, as exemplified by the label changes called for by the FDA outlining its contribution to *UGT1A1* pharmacogenetics. This also has implications for irinotecan, nilotinib, and indacaterol prescribing. In the present investigation, a well-known single nucleotide polymorphism (SNP), *rs4148325*, is studied to find its association or lack of it with serum bilirubin, AST, and ALT levels in patients taking simvastatin.

Methods & Materials

Our patent cohort comprised male and female patients between 36 and 67 years. The sample size was 110. Patients who were taking simvastatin for the treatment of hyperlipidemia were part of the study. Only a few patients were taking simvastatin before the start of this investigation.

For the duration of this investigation (4 weeks), patients were on a 20 mg PO qDay dosing schedule. Blood levels of bilirubin, ALT, and AST were measured before and after the study period (on day 1 and day 28). A 2ml blood was also taken in EDTA-containing vacutainers for the DNA analysis. The study was performed per the Ethical Guidelines of COMSATS University Islamabad, Abbottabad Campus, and all patients were verbally informed about the study and provided written informed consent before being part of this investigation.

Genotype Analysis

DNA was extracted from the samples obtained in the EDTA-containing vacutainers as per the standard manual procedures. A single SNP (*rs4148325*) was investigated. The DNA section of interest was amplified by PCR and then gel purified. Purified PCR products were sent to a commercial sequencing facility in China. The sequences were returned within two weeks, which were then analyzed for quality and variant calling.

Table 1 Genotype distribution of the investigated cohort in male and female genders.

Gender * Genotype					
Count					
		Genotype			Total
		CC	TC	TT.	
Gender	Male	21	14	11	46
	Female	29	23	12	64
Total		50	37	23	110

Statistical Analysis

Version 26.0 of the IBM Statistical Package for Social Sciences SPSS was used for data-keeping and analysis. Mean ± SD was used to represent continuous variables, whereas discrete variables were represented as percentages and frequencies. A Chi-square/ANOVA/ t-test test was used to compare genotype groups' bilirubin, ALT, and AST levels. A p-value of 0.05 or less was regarded as statistically significant.

Results

The mean age of the investigated cohort was 50.08±8.35, the mean height was 10.99±835, and the mean weight was 67.64±7.54. There were more female patients (64) than male (46). Most patients were non-smokers (92), while smokers were only 18. Wild type genotype (CC) was found in 50 patients; 37 patients had the CT genotype, while the TT genotype was found in only 23 patients. Among the patients with the CC genotype, 21 were male, while 29 were female. Of patients with the CT genotype, 14 were male, and 23 were female. Similarly, in patients with the TT genotype, 11 were male, and 12 were female (Table 1). Strangely, among the smokers, females (12) were more than males (6) (Table 2). Among the smokers, 9 had the CC genotype, 3 had the CT genotype, and 6 patients had the TT genotype (Table 3).

Table 2 Smoking status of the investigated cohort in terms of the male and female genders.

Gender * Smoking Status				
Count				
		Smoking Status		
		No	Yes	Total
Gender	Male	40	6	46
	Female	52	12	64
Total		92	18	110

Table 3 Genotype distribution of the investigated cohort in terms of smoking status.

Genotype * Smoking Status				
Count				
		Smoking Status		Total
		No	Yes	
Geno-type	CC	41	9	50
	TC.	34	3	37
	TT.	17	6	23
Total		92	18	110

The mean difference in bilirubin level between days 1 and 28 in the CC group was 0.21 mg/dl; in the CT group, it was 0.19 mg/dl; and in the TT group, it was 0.20 mg/dl (Table 4). Although there was a decrease in the bilirubin levels in the CT group, the increase was statistically insignificant (p=0.62, Table 4). Similarly, the mean difference in AST levels between days 1 and 28 in the CC group was 39.04 U/L; in the CT group, it was 34.62 U/L; and in the TT, it was 37.95 U/L. Again, the difference was least in the CT group, but there was no statistically significant difference between the groups (p=0.32, Table 5). For ALT, the mean difference between days 1 and 28 in the CC groups was 36.34 U/L; in the CT group, it was 35.78 U/L; and in TT, it was 30.30 U/L. There was a large decrease in ALT levels in the TT group; however, that difference did not reach a significant level (p=0.30, Table 6).

Table 4 Mean difference in bilirubin levels between days 1 and 28 in CC, CT, and TT genotypes.

Groups	*Count*	*Sum*	*Average*	*Variance*
CC	50	10.66	0.2132	0.006324245
TC	37	7.29	0.197027027	0.006088138
TT	23	4.67	0.203043478	0.005385771

Discussion

In patients who were given simvastatin, a statistical association between genotypic polymorphs of the *UGT1A1* gene and elevated levels of bilirubin, AST, and ALT could not be observed. The *UGT1A1* gene encodes the enzyme called UDP-glucuronosyltransferase 1-1, which is solely responsible for metabolizing bilirubin (Barbarino et al. 2014). The mean difference in bilirubin level between days 1 and 28 was reduced in the patients with CT genotype but did not reach statistical significance (p=62). Similarly, AST levels were also reduced in CT groups and, to some extent, in the TT group; however, the difference was not statistically significant (0.32). For AST, patients in the TT group showed the highest reduction, but it was not statistically significant (p=0.30).

Table 5 Mean AST level differences between days 1 and 28 in CC, CT, and TT genotypes.

Groups	*Count*	*Sum*	*Average*	*Variance*
CC	50	1952	39.04	202.1616327
TC	37	1281	34.62162162	205.5195195
TT	23	873	37.95652174	127.5889328

Table 6 Mean difference in ALT levels between days 1 and 28 in CC, CT, and TT genotypes.

Groups	*Count*	*Sum*	*Average*	*Variance*
CC	50	1817	36.34	223.78
TC	37	1324	35.78378378	314.7297297
TT	23	697	30.30434783	226.8577075

UGT1A1 (rs4148325) single nucleotide polymorphisms (SNPs) have been reported to be associated with bilirubin level variations (Johnson et al. 2009). Disease-Modifying-Antirheumatic-Drugs (DMARDs), such as methotrexate, have been known to enhance conjugated bilirubin levels. The administration of methotrexate in pediatric leukemia has also been found to increase direct and total bilirubin levels (Bordbar et al. 2018). Findings in patients treated with tocilizumab, another Interleukin 6 receptor alpha (IL-6Rα) inhibitor, are in line with that of our study, which indicates that IL-6 pathway inhibition leads to decreased UDP-glucuronosyltransferase 1-1 activity in Gilbert's syndrome patients(Lee et al. 2011, Mori, Terada, and Ueki 2012). Even though similar findings in investigations of two IL-6Rα lead to the inference of a class effect, the exact mechanism of saturation or inhibition of UDP-glucuronosyltransferase 1-1 remains a mystery. In rat hepatocytes, researchers have shown that *UGT1A1* mRNA levels are affected by IL-6 (STRASSER, MASHFORD, and DESMOND 1998). IL-6R blockade, as per an alternate hypothesis, is believed to correct hypoferremia caused by hepcidin (Ganz 2019). Subsequently, in patients harboring *UGT1A1* mutations, enhanced hemoglobin turnover leads to saturated renal clearance due to an increase in unconjugated bilirubin. The exact mechanism for bilirubin elevations in Gilbert's Syndrome patients is yet to be determined. Placebo+DMARD-treated patients had less

frequent ALT/AST elevations as compared sarilumab + DMARD-treated patients according to results obtained from clinical trials (Genovese et al. 2015).

Direct glucuronidation of several drugs or their metabolites, including sarilumab, ibuprofen, and irinotecan, is because of the involvement of *UGT1A1 (Strassburg 2008).* Variants on the *UGT1A1* gene have exhibited associations with treatment-related adverse effects in patients given irinotecan and atazanavir. Post-treatment with irinotecan patients who are homozygous for the *UGT1A1*28* allele have a greater risk of suffering from neutropenia (Fujita and Sparreboom 2010). These adverse events are due to the inhibited glucuronidation and clearance of SN38, an active metabolite of irinotecan. The neutropenia is observed to not be directly related to the metabolism of bilirubin, nor does it have any similarities with mild elevations in bilirubin seen in simvastatin-treated patients. The neutropenia is solely directly related to SN-38 activity in irinotecan-treated patients. Some patients show signs of hyperbilirubinemia post administration of Atazanavir, with 40% reporting bilirubin levels >2.5 × Upper Limit of Normal (ULN) referred to as Grade 3, and ≈4 to 8% reporting bilirubin levels ≥5 × ULN (Grade 4) (Gammal et al. 2016). As compared to the mild bilirubin elevations seen in patients who are given simvastatin, more frequent cases and much higher levels of hyperbilirubinemia are seen in Atazanavir treated patients. Only a small portion of simvastatin-treated patients had bilirubin elevations >1.5 × ULN and none had Grade 3 or 4 hyperbilirubinemia.

As per our findings, genotypic variations in the UGT1A1 gene are not responsible for simvastatin-related increases in bilirubin, AST, and ALT levels. Gilbert's Syndrome patients that are already predisposed to lower bilirubin metabolism are more susceptible to simvastatin-invoked mold bilirubin elevations. However, fusing data obtained from genotyping pertaining to safety assessments, more specifically to enhancements in bilirubin levels, can allow for accurate diagnoses of liver safety events in patients who are administered simvastatin.

Conflict of Interest

The authors declare that they have no competing interests. None of the authors is expected to gain financially from the results of this study.

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The experiments in this investigation were carried out using internal resources of the authors. There was no specific funding available for this project.

Study Approval

Yes. The study was approved by the Institutional Review Board & Ethics Committee of the COMSATS University Islamabad, Abbottabad Campus

Consent Forms

Patients in this study were briefed about the project and each participant signed a consent form. These consent forms signed by the patients are available with the authors.

Authors Contribution

MNR conceptualized the study and wrote the final manuscript, RUS and HUR collected samples, carried out the experimental work, helped with the analysis and writing the first draft, SN performed the statistical analysis, and MNR supervised the whole project.

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