Pharmacogenetic Testing Can Identify Patients Taking Atazanavir at Risk for Hyperbilirubinemia

To the Editors:

In 2012, there were approximately 4.7 million people living with HIV in Asia and approximately 1.25 million in the region accessing combination antiretroviral treatment (cART).[1] With longer duration of cART treatment, increasing numbers of patients will be switched from first- to second-line cART. Recommended second-line regimens are ritonavir-boosted atazanavir (ATV/r) or lopinavir (LPV/r) together with 2 nucleoside reverse transcriptase inhibitors, one of which should be zidovudine or tenofovir.[2] Advantages of ATV/rover LPV/r include convenient once-daily dosing and minimal effect on lipid profiles, but higher plasma concentrations correlate with hyperbilirubinemia as a consequence of inhibition of the uridine-glucuronosyltransferase (UGT) 1A1 enzyme responsible for bilirubin conjugation.[3]

Polymorphisms in the *UGT1A1* gene influence the risk of hyperbilirubinemia. Six thymine adenine repeats (TA6) are found in the promoter region of the wild-type allele. A less common variant containing 7 repeats (TA7) results in lower promoter activity. The presence of at least 1 TA7 allele has been significantly associated with grade 3–4 hyperbilirubinemia in whites.[4]

We previously reported that Thai subjects taking ATV/r 200/100 mg QD showed equivalent plasma concentrations to white subjects taking standard dose (300/100 mg). The prevalence of grade 3 and 4 hyperbilirubinemia fell significantly from 36% to 14% after subjects were switched to the reduced dose,[5] and reduced dose ATV/r demonstrated virological efficacy in a small study conducted in Thai patients over a median of 68 weeks.[6]

The P-glycoprotein (P-gp) membrane transporter, encoded by the *multidrug resistance 1* (*MDR1*) gene, influences intracellular and plasma ATV concentrations. *MDR1* gene polymorphisms have been shown to influence ATV plasma concentrations. At least 1 polymorphic nucleotide change at position 3435 (3435C>T) resulted in significantly lower ATV trough concentrations (C_{trough}), but no difference in viral load reduction after 12 weeks of treatment in subjects initiating treatment with ATV/r-based cART.[7]

The extent to which polymorphisms in the *MDR1* and *UGT1A1* genes influence the plasma concentrations and prevalence of hyperbilirubinemia in Asian patients has not been described. In this study, we describe the prevalence of nucleotide polymorphisms in these genes in Thai patients taking ATV/r, and their relationship to ATV C_{trough} and serum bilirubin concentrations.

All subjects taking ATV 200 mg or 300 mg and 2 nonnucleoside reverse transcriptase inhibitors had genotypes assessed, and ATV concentrations assessed using HPLC as previously described.[5] C_{trough} concentrations were obtained 24 hours after the previous dose was taken.

Eighty-eight patients (47% female) with median (interquartile range) age 41 (36–47) years were studied: 24 (27%) taking ATV/r 200/100 mg QD, 35(40%) taking ATV/r 300/100 mg QD, and 29 (33%) taking ATV/r initially at 300/100 mg QD who thereafter had their dose reduced to 200/100 mg QD had *MDR1* and *UG1A1* genotypes assessed. Patients who were taking ATV/r reduced dose switched from the higher to the lower dose for at least 4 weeks before measurement of the C_{trough}.

After written informed consent was obtained, 3 mL of peripheral blood was drawn and genomic DNA extracted from peripheral blood mononuclear cells using Qiagen DNA extraction kits (Qiagen, Valencia, CA). To determine the numbers of the TA repeats in the *UGT1A1* promoter, a pair of primers, 5-FAM (carboxyfluorescein)-labeled forward primer (5′-CACGTGACACAGTCAAAC-3′) and unlabeled reverse primer (5′-CAACAGTATCTTCCCAGC-3′), was used to polymerase chain reaction (PCR) amplify its promoter region.[8] Then, PCR products were electrophoresed and their sizes determined by GeneMapper version 4.0 software (Applied Biosystems, Carlsbad, CA). The genotype of *MDR1* nucleotide position 3435 was assessed by PCR-restriction fragment length polymorphism using primers and restriction enzyme, *Dpn*II, as previously described.[9]

UGT1A1 genotype was not available in 1 subject. The distribution of *UGT1A1* genotypes was 65 (75%) common homozygotes (TA_6/TA_6), 22 (25%) heterozygotes (TA_6/TA_7), and there were no rare homozygotes (TA_7/TA_7). The distribution of *MDR1* 3435C>Tgenotypes was 28 (31%) common homozygotes (CC), 45 (51%) heterozygotes (CT), and 15 (17%) rare homozygotes (TT).

Statistical comparisons between groups were made using a Wilcoxon test for continuous data, and Fisher exact test for categorical data. Median (interquartile range) ATV C_{trough} and total bilirubin concentrations at both ATV doses, by *MDR1* genotypes with 1 T allele and *UGT1A1* genotype are shown in Table 1. Although ATV C_{trough} concentrations were lower in those with at least 1 T allele in position 3435 of the *MDR1* gene taking ATV/r 300/100 mg versus common homozygotes, no significant differences in ATV C_{trough} concentrations were noted in patients with at least 1 T allele in either dose group. Likewise, no significant differences in bilirubin concentrations were noted between *UGT1A1* homozygotes and heterozygotes in either dose group. However, patients taking ATV/r 200/100 mg with a TA_6/TA_7 genotype had a significantly higher prevalence of grade 3–4 hyperbilirubinemia compared with common homozygotes [4/15 (27%) versus 2/37 (5%), respectively, $P = 0.05$]. No difference in the prevalence of grade 3–4 hyperbilirubinemia was noted in heterozygotes versus common homozygotes

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TABLE 1. Median (IQR) ATV Trough Concentrations, and Total Serum Bilirubin Concentrations in Patients by Genotype and ATV/r Dose Regimen

	ATV/r 200/100 mg QD			ATV/r 300/100 mg QD		
	MDR1 CC	MDR1 CT/TT	*P*	MDR1 CC	MDR1 CT/TT	*P*
Median (IQR) ATV C_{trough} (mg/L)	0.59 (0.35–0.95)	0.57 (0.38–1.11)	0.7	1.19 (0.70–2.22)	0.75 (0.49–1.5)	0.15
Median (IQR) serum bilirubin (mg/dL)	1.7 (1.2–2.6)	1.5 (0.8–2.4)	0.5	2.4 (1.6–3.6)	1.8 (1.5–2.6)	0.10
	UGT1A1 TA_6/TA_6	UGT1A1 TA_6/TA_7		UGT1A1 TA_6/TA_6	UGT1A1 TA_6/TA_7	
Median (IQR) ATV C_{trough} (mg/L)	0.59 (0.36–1.11)	0.56 (0.38–0.97)	1.0	0.92 (0.6–2.02)	0.68 (0.48–1.27)	0.2
Median (IQR) serum bilirubin (mg/dL)	1.6 (0.9–2.3)	1.9 (0.9–2.3)	0.3	2.0 (1.5–2.7)	1.9 (1.5–3.1)	0.9

IQR, interquartile range.

was noted in subjects taking standard dose ATV/r [4/16 (25%) versus 8/48 (17%), respectively, $P = 0.48$].

Although the frequencies of the *UGT1A1* TA7 allele in Thai (15.6%)[10] and Asians (11.6%–14.5%) are approximately half of those in whites (26.0%–33.2%),[11] hyperbilirubinemia is a common problem in Thai patients taking ATV/r. Although not life threatening, it is extremely disturbing to patients and heightens stigma. *UGT1A1* genotype testing is a useful tool to determine which patients might have an elevated risk of hyperbilirubinemia, even after being treated with a reduced dose of ATV/r.

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Similar Success Rates but Lower Incidence of Telaprevir-Related Rash in HIV/HCV Coinfected as Compared to HCV-Monoinfected Patients Treated With Triple Anti-HCV Therapy

To the Editors:

INTRODUCTION

After the introduction of first-generation direct acting antivirals, boceprevir and telaprevir (TVR), in the treatment armamentarium of genotype-1 hepatitis C virus (HCV) chronic infection, high rates of sustained virological response (SVR) are now commonly seen in clinical practice, ranging from 51% to 75%.[1–4]

Triple regimens with pegylated interferon alfa (PEG-IFNα), ribavirin (RBV), and the protease inhibitor TVR have been widely studied in HCV-infected patients, and to a lesser extent, in subjects with HIV coinfection.[5–8]

The study was a retrospective analysis of data generated by routine clinical practice funded by the national health system.
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