Toward a Pharmacogenetic Understanding of Nucleotide and Nucleoside Analogue Toxicity

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(See the article by Izzedine et al., on pages 1481–91.)

Antiretroviral therapies for HIV-1 infection have become progressively more potent and better tolerated. An important milestone was the 2001 Food and Drug Administration approval of tenofovir disoproxil fumarate (TDF), a once-daily nucleotide analogue reverse-transcriptase inhibitor that is available commercially as a single agent (Viread; Gilead Sciences), coformulated with emtricitabine (Truvada; Gilead Sciences), and coformulated with emtricitabine and efavirenz (Atripla; Gilead Sciences and Bristol-Myers Squibb). Compared with some nucleoside analogues, TDF is notable for less mitochondrial toxicity in vitro and in animal models [1, 2] and for lower rates of nucleoside analogue–associated toxicities, such as lipatrophy and peripheral neuropathy [3, 4]. As with all antiretroviral agents, however, TDF use has been associated with serious toxicity in some individuals. In particular, TDF has been infrequently associated with renal tubular injury. This was not completely unexpected in light of prior experience with structurally related compounds. The nucleotide antiviral drug adefovir dipivoxil is safe at low doses for the treatment of hepatitis B virus infection but was associated with proximal renal tubular injury when studied at higher doses [5], precluding its use for treatment of HIV infection. The anti-cytomegalovirus nucleotide cidofovir also causes renal toxicity [6, 7].

On the basis of the adefovir experience, there was enhanced monitoring for renal adverse events during clinical trials of TDF in HIV-infected volunteers. These trials [3, 4, 8] and at least one postmarketing observational study [9] did not demonstrate increased rates of renal dysfunction with TDF. Subsequent case series and cohort studies, however, have implicated TDF as a cause of renal dysfunction that ranges from mild, reversible renal insufficiency [10] and Fanconi syndrome [11, 12] to more-severe renal failure [13]. Characteristic features include decreased creatinine clearance with increased urinary wasting of phosphate, glucose, calcium, uric acid, and, in some cases, protein. Factors that may increase an individual’s risk for this toxicity include preexisting renal impairment, lower body weight, and concomitant use of nephrotoxic drugs or HIV protease inhibitors, in particular ritonavir [14–16]. Exclusion of individuals with preexisting renal impairment from clinical trials of TDF [3, 4] may, in part, explain the infrequency of TDF-associated renal dysfunction in these studies.

There is considerable interindividual variability in the likelihood, character, and severity of renal dysfunction with TDF, suggesting a possible influence of host genetics on susceptibility. In this issue of the Journal, Izzedine et al. [17] provide the first report of a possible association between a human genetic variant and TDF-associated renal dysfunction. This finding may be very important, assuming that the associations identified are confirmed in independent studies. Izzedine et al. defined renal proximal tubulopathy on the basis of the presence of metabolic acidosis, renal potassium and amino acid loss, and low serum phosphorus and uric acid levels. This outcome was significantly associated with a single G→A substitution at position 1249 of ABCC2 (also called “MRP2,” which encodes multidrug resistance protein [MRP] 2) and with an ABCC2 haplotype comprising 4 polymorphisms, including 1249 G→A (unadjusted odds ratio, 4.25; lower bound of 95% confidence interval, 1.25). Additionally, a T→A substitution at position 3563 and a
haplotype including this polymorphism were significantly associated with the absence of renal toxicity (none of the case patients had these variants). A synonymous polymorphism (i.e., one that does not alter the amino acid sequence of the encoded protein) in ABCC4 (also called “MRP4,” which encodes MRP4) was also associated with the outcome ($P = .04$) and was included as a covariate in the multivariable analyses of the ABCC2 haplotypes. Izzedine et al.’s report highlights both the promise and the limitations of genetic association studies related to HIV drug toxicity.

It is certainly biologically plausible that polymorphisms in the renal tubular drug transporter genes studied by Izzedine et al. would influence the disposition of TDF and could, therefore, affect the risk of renal toxicity. Of the many (at least 15) drug transporters identified to date in the proximal renal tubule [18], both MRP2 and MRP4 have been suggested to transport TDF [19, 20]. Biological plausibility is important for candidate gene association studies, although the optimal quantity and quality of data needed to buttress this plausibility are not known. It is not clear why Izzedine et al. included ABCB1 (also called “MDR1,” which encodes P-glycoprotein) in their study, because TDF is not known to be transported by P-glycoprotein. This was not unreasonable, however, given reported associations between ritonavir (a known P-glycoprotein inhibitor) and TDF-associated renal toxicity. As with many nucleoside analogue toxicities, manifestations of TDF-associated renal toxicity are heterogeneous and do not have universally accepted diagnostic criteria, which raise the possibility of misclassification of case patients and control subjects. Izzedine et al.’s study benefited from the use of a standard prospective screening protocol for all TDF recipients, which should have minimized misclassification. Older age and (consistent with previous reports) concomitant protease inhibitor use were more common among persons who developed renal toxicity. Although adjustment for these factors did not diminish the association with ABCC2, the relative contribution of host genetics to this toxicity may be better quantified by a study in which TDF recipients are matched for these and other putative risk factors.

The ABCC2 polymorphisms described in Izzedine et al.’s study are nonsynonymous (i.e., they alter the amino acid sequence of the encoded protein), but little is known about their functional effects. Mechanisms by which these variants might increase susceptibility to drug toxicity are, therefore, speculative. Their study involved a remarkably small number of case patients ($n = 13$) and control subjects ($n = 17$), all but 1 of whom were white French individuals. Although a small sample size inevitably reduces the power to detect a true difference in the relative frequency of the outcome between groups (type II error), a positive finding should not be viewed with skepticism solely because of sample size. However, as with many genetic association studies, the statistical analyses used place the study at risk for false discovery due to multiple comparisons (i.e., these associations may be observed by chance alone) [21]. The $P$ values reported would not remain significant at $P < .05$ if corrected for multiple comparisons. Guidelines have recently been proposed for statistical approaches to genetic association studies [21, 22]. Because large numbers of polymorphisms are now routinely investigated in such studies, very few associations could withstand traditional multiple comparison corrective approaches. Emphasis should, therefore, be placed on validating initial associations, including the one reported by Izzedine et al., in separate studies.

Large-scale genetic association studies of the influence of human genetics on the response to antiretroviral therapy have been made feasible by DNA banks linked to prospective HIV clinical trials [23] and cohort studies [24]. Recent reports have contributed to our understanding of the pharmacogenetics of antiretroviral therapy, but most have focused on nonnucleoside reverse-transcriptase inhibitors and protease inhibitors, in part because their active moieties are readily quantifiable in plasma and because there is a long history of the study of functional variants of hepatic cytochromes that metabolize these drugs. In contrast, nucleoside and nucleotide analogues require intracellular phosphorylation to their virologically active anabolites. Because the processing of specimens for precise measurement of intracellular nucleoside triphosphates (or nucleotide diphosphates) is technically demanding—and because therapeutic (or toxic) concentrations are not well characterized—there are fewer data on the intracellular pharmacodynamics of these compounds. Additionally, nucleoside analogue toxicities such as lipodystrophy and peripheral neuropathy are often diagnosed subjectively in the clinic and may require expensive imaging or invasive testing for definitive evaluation. Even the diagnosis of hyperlactatemia, which should be readily detected by routine phlebotomy, is technically challenging, with high false-positive rates and uncertain clinical significance [25].

In part because of these limitations, there have been relatively few genetic association studies focused on nucleoside analogue toxicities. These have tended to involve heterogeneous genes and outcomes, often have small sample sizes, and some initial associations have not yet been validated. To date, published studies have sought to identify genetic predictors of abacavir hypersensitivity [26, 27], lipodystrophy [28–30], pancreatitis [31], and peripheral neuropathy [32, 33]. Only associations between abacavir hypersensitivity and HLA type [34, 35] and between lipodystrophy and a tumor necrosis factor–α gene promoter polymorphism [28, 29] have been confirmed in $>1$ study. Furthermore, only for abacavir hypersensitivity has genetic testing been applied in clinical practice [36] and subjected to cost-effectiveness analysis [37].

Of the various antiretroviral drug classes, only nucleoside/nucleotide analogues
References


