Drug Transporter and Metabolizing Enzyme Gene Variants and Nonnucleoside Reverse-Transcriptase Inhibitor Hepatotoxicity

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This nested case-control study examined relationships between MDRI, CYP2B6, and CYP3A4 variants and hepatotoxicity during antiretroviral therapy with either efavirenz- or nevirapine-containing regimens. Decreased risk of hepatotoxicity was associated with MDRI 3435C>T (odds ratio, 0.254; P = .021). An interaction between MDRI and hepatitis B surface antigen status predicted risk with 82% accuracy (P < .001).

The nonnucleoside reverse transcriptase inhibitors (NNRTIs) nevirapine and efavirenz are widely used to treat HIV-1 infection, but adverse reactions are common. The risk of nevirapine-associated hepatotoxicity is greatest in patients with high CD4+ counts [1]. Efavirenz is less likely than nevirapine to cause hepatotoxicity in patients with high CD4+ counts [2], but at lower CD4+ counts (<250 cells/mm3 for women and <400 cells/mm3 for men), the frequency of symptomatic hepatic events is comparable to that of symptomatic hepatic events associated with nevirapine [1].

There is considerable interindividual variability in the metabolism and disposition of nevirapine and efavirenz, at least some of which is because of human genetic differences. Both drugs are metabolized primarily by cytochrome P450 (CYP) 2B6, and functional differences between genetic variants have been identified. A CYP2B6 G>T polymorphism at position 516 is associated with higher plasma exposure for efavirenz [3] and nevirapine [4]. In addition, CYP3A isoforms have been implicated in NNRTI metabolism.

A previous study [5] suggested that P-glycoprotein (encoded by MDRI, which is also called ABCB1) transports neither nevirapine nor efavirenz. However, intracellular nevirapine concentrations correlate inversely with peripheral blood mononuclear cell P-glycoprotein expression [6], and MDRI 3435C>T has been associated with more-favorable virologic responses to efavirenz-containing regimens [3, 7]. The role of P-glycoprotein in NNRTI disposition remains controversial. We examined whether polymorphisms in CYP2B6, CYP3A4, and MDRI were associated with risk of NNRTI hepatotoxicity among patients prescribed antiretroviral regimens that included either nevirapine or efavirenz.

Materials and methods. This study included HIV-infected individuals who initiated their first NNRTI-containing regimen (either nevirapine or efavirenz) between January 1998 and November 2003 while receiving HIV care at the Comprehensive Care Center in Nashville, Tennessee. Eligible subjects had levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) documented at baseline (before they initiated NNRTI-containing regimens), had subsequent ALT and AST level determinations during treatment with an NNRTI, and previously had consented to specimen storage for genetic testing. The definition for hepatotoxicity was the occurrence of at least 1 increase in the serum ALT or AST level >5 times the upper limit of the normal range during NNRTI therapy in an individual with baseline values <3 times the upper limit of the normal range. Laboratory evaluations were obtained during routine clinical care. Specimens for drug concentration assays were unavailable.

A nested case-control study design was used. Case patients developed hepatotoxicity while being treated with an NNRTI-containing regimen, whereas controls did not develop hepatotoxicity while being treated with an NNRTI-containing regimen. Multiple controls were identified for each case patient. Case patients and controls were matched for the specific NNRTI, race, age (within 5 years), and seropositivity for hepatitis C virus antibody. This study was approved by the Vanderbilt University Medical Center Institutional Review Board.

Robotic DNA extraction was performed on cryopreserved peripheral blood buffy coats. Genotyping was performed by TaqMan 5′-exonuclease assay (Applied Biosystems; CYP2B6...
Baseline characteristics were compared using Fisher’s exact, Student’s t test, or the Wilcoxon rank-sum test. Fisher’s exact test and logistic regression were used to test for genetic association. Gene-gene and gene-environment interactions associated with hepatotoxicity were detected by multifactor dimensionality reduction. This computation-intensive method, which is described elsewhere [8], identifies composite groups of genotypes and/or environmental factors that distinguish groups that are at high risk from those at low risk with respect to outcome. This approach can identify predictive models in the absence of main effects for individual factors.

Results. Of 423 patients who initiated nevirapine- or efavirenz-containing regimens, 201 (48%) initiated nevirapine and 222 (52%) initiated efavirenz. Twenty patients (4.7%) experienced severe hepatotoxicity, including 14 nevirapine recipients and 6 efavirenz recipients. Of the 20 patients who experienced severe hepatotoxicity, 13 (65%) had provided consent for genetic analyses (9 nevirapine recipients and 4 efavirenz recipients). Forty-nine matched controls were identified. Subsequent analyses involved these 62 individuals, unless stated otherwise. Case patients and controls did not differ with respect to specific NNRTI, median age (37.5 years), race (80% white and 18% black), or hepatitis C seropositivity. In addition, there were no significant differences with respect to median baseline CD4+ count (236 cells/mm3), HIV-1 RNA level (4.9 log10 copies/mL), body mass index (23.4), or albumin level (4.1 g/dL). Case patients were more likely than controls to be male (100% and 71.4%, respectively; P = .03) and to have baseline ALT levels >40 IU/L (61.5% and 25.5%, respectively; P = .03). Of the 13 case patients, 2 (15.4%) had rash, 3 (23.1%) had fever, and an additional 2 (15.4%) had both rash and fever associated with hepatotoxicity. Hepatotoxicity was documented a median of 427 days (IQR, 187–779 days; ). The longer exposure to the NNRTI, median age (37.5 years), race (80% white and 18% black), or hepatitis C seropositivity. In addition, there were no significant differences with respect to median baseline CD4+ count (236 cells/mm3), HIV-1 RNA level (4.9 log10 copies/mL), body mass index (23.4), or albumin level (4.1 g/dL). Case patients were more likely than controls to be male (100% and 71.4%, respectively; P = .03) and to have baseline ALT levels >40 IU/L (61.5% and 25.5%, respectively; P = .03). Of the 13 case patients, 2 (15.4%) had rash, 3 (23.1%) had fever, and an additional 2 (15.4%) had both rash and fever associated with hepatotoxicity. Hepatotoxicity was documented a median of 427 days (IQR, 187–779 days; P = .03). The longer exposure time among case patients reflects continued therapy in some individuals, despite elevations in transaminase levels. By univariate analysis, the MDRI position 3435 T allele was associated with a decreased likelihood of hepatotoxicity (OR, 0.25; 95% CI, 0.09–0.76; P = .021). There were no independent main effects for CYP2B6 1459C→T, CYB2B6 516G→T, or CYP3A4 -392A→G (P > .1). Case patients and controls did not differ with respect to concomitant use of protease inhibitors, alcohol, or cocaine. Case patients were more likely than controls to be positive for hepatitis B surface antigen (HBsAg; OR, 21.5; 95% CI, 2.1–218.3; P = .01). Analyses were repeated after including 5 individuals (4 case patients and 1 control) with baseline transaminase levels between 3 and 5 times the upper limit of the normal range. An association between MDRI 3435C→T and a decreased likelihood of hepatotoxicity was still observed (OR, 0.44; P = .04). The sample size was not sufficient to analyze nevirapine and efavirenz separately.

Multifactor dimensionality reduction analysis was performed to identify gene-gene interactions related to hepatotoxicity risk. Because the dataset was unbalanced, case patients were oversampled to achieve balance. Discussion of oversampling is provided elsewhere [9]. An interaction between MDRI 3435C→T and CYP2B6 1459C→T correctly predicted hepatotoxicity status 74% of the time (P < .001). Risk was decreased in subjects with at least 1 MDRI 3435 T allele, unless the individuals were also CYP2B6 1459 T T homozygotes (figure 1A). To explore gene-environment interactions, multifactor dimensionality reduction analysis was repeated to include sex, baseline CD4+ count (dichotomized at the median count), prior elevation in ALT level (dichotomized at 40 IU/L), and HBsAg positivity. An interaction between MDRI 3435C→T and HBsAg positivity correctly predicted hepatotoxicity 82% of the time (P < .001) (figure 1B). When these analyses were repeated after including the additional 5 individuals with elevated baseline ALT or AST levels, interactions were still identified between MDRI 3435C→T and CYP2B6 1459C→T (P < .001) and between MDRI 3435C→T and HBsAg positivity (P < .001).

Discussion. A C→T polymorphism at MDRI position 3435 was significantly associated with decreased risk of hepatotoxicity with NNRTI-containing regimens. Hepatotoxicity risk category was predicted with 74% accuracy by an interaction between MDRI 3435C→T and CYP2B6 1459C→T. An interaction involving CYP2B6 is not unexpected, because NNRTIs are metabolized by CYP2B6, and 1459C→T has been associated with decreased hepatic CYP2B6 expression. However, plasma efavirenz and nevirapine exposure are predicted by CYP2B6 516G→T, not by 1459C→T [3, 4]. Therefore, this interaction between MDRI and CYP2B6 C1459T should be considered tentative. Hepatotoxicity risk was also predicted with 82% accuracy by an interaction between MDRI C3435T and HBsAg positivity. The favorable association with MDRI 3435 T heterozygosity was lost in the presence of HBsAg.

The MDRI gene encodes P-glycoprotein, an efflux transporter that eliminates many drugs from cells and tissues. Overexpression of P-glycoprotein protects tumor cells against cytotoxic effects of structurally diverse anticancer agents. Although some data support a relationship between MDRI C3435T and P-glycoprotein expression, findings have been inconsistent [10]. At present, the relationships between MDRI polymorphisms and hepatic P-glycoprotein functional activity remain uncertain.

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516G→T, CYP2B6 1459C→T, and CYP3A4 -392A→G) or allele-specific oligonucleotide ligation (MDRI C3435T).

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therapy [1] have led to changes in prescribing recommendations. Additional reported risk factors include hepatitis B or hepatitis C coinfection, baseline elevations in hepatic transaminase level, and low body mass index [2, 11]. In the present study, NNRTI-associated hepatotoxicity was associated with hepatitis B coinfection and elevated transaminase levels at baseline, which is consistent with previous reports. We could not assess the effect of hepatitis C seropositivity, because this was a matching variable. In contrast to previous reports, case patients were somewhat more likely to be male, and we did not find associations with higher baseline CD4+ count or lower body mass index.

The occurrence of rash and/or fever associated with hepatotoxicity in at least some nevirapine recipients during the first 6 weeks of treatment suggests immune mechanisms, and a recent study involving 14 nevirapine reactions suggested increased susceptibility with HLA-DRB1*0101 [12]. However, absence of rash or fever in many case patients suggests that NNRTI-associated hepatotoxicity is not always immune mediated. Multiple mechanistic pathways may be involved.

This study was limited by its small sample size. However, validity is supported by a separate study that shows this association between MDR1 3435C→T and nevirapine hepatotoxicity [13]. Matching case patients and controls for important parameters increased our ability to identify associations. Although we analyzed together individuals treated with either nevirapine or efavirenz, we believe that doing so was justified, given the pharmacologic similarities between these drugs, including metabolism primarily by CYP2B6 (although metabolism and transport may differ in other ways). A larger study could allow identification of more-complex gene-gene and gene-environment interactions.

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