

Genetic Variation in Drug Transporter and Metabolizing Enzyme Genes May be Associated with Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) Hepatotoxicity

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Background

- Non-nucleoside reverse transcriptase inhibitors (NNRTI) nevirapine (NVP) and efavirenz (EFV) can cause hepatotoxicity in some patients
- Genetic predictors of NNRTI hepatotoxicity are uncertain
- Identifying persons at increased risk of hepatotoxicity would be beneficial, so that optimal treatment regimens could be utilized
- We hypothesize that genetic variation in drug metabolizing and transporter genes may be relevant predictors of hepatotoxicity risk during NNRTI treatment

Background

- NVP and EFV are metabolized primarily by hepatic cytochrome P450 (CYP) 2B6
- *CYP2B6* G516T is associated with increased plasma EFV levels
- P-glycoprotein, encoded by *MDR1*, affects hepatic transport of many compounds
- To explore possibility that drug metabolizing enzymes and transporter genes are related to risk of NNRTI hepatotoxicity, we characterized associations between candidate gene SNPs and hepatotoxicity in patients treated with NVP or EFV

Methods

- Study subjects were receiving HIV care at the Comprehensive Care Center (CCC)
- NVP or EFV prescribed during routine clinical care
- A nested case-control study was conducted among a cohort of 445 HIV+ adults who initiated their first NNRTI treatment at the CCC
- Eligible subjects had liver function tests available prior to initiating therapy, and had follow-up data
- Peripheral blood buffy coats were used for DNA isolation
- DNA extractions were conducted using robotic DNA extraction on an AUTOPURE LS[®] instrument (Gentra Systems, Minneapolis, MN)

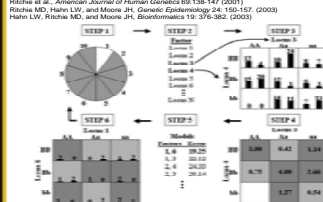
Methods

- Case definition: AST or ALT >5x upper limit of normal or bilirubin >3.5 on NNRTI therapy – 28 patients (6.3%); 20 had DNA available
- Control definition: did not develop hepatotoxicity – matched to cases on NNRTI, age (+/- 5 years), race, and hepatitis C status
- Polymorphisms identified: *CYP2B6* G516T and C1459T, *CYP3A4* A-392G, and *MDR1* C3435T
- Genotyping conducted using TaqMan and OLA technologies
- Statistical analyses conducted using SAS V9.1 and Multifactor Dimensionality Reduction software

Multifactor Dimensionality Reduction

- Step 1: Split data for cross validation.
- Step 2: Choose a set of genetic polymorphisms.
- Step 3: Calculate a ratio between the number of cases and controls in each multifactor class in the training data set.
- Step 4: Label each multifactor cell as high-risk or low-risk based on some threshold (#cases / #controls > 1.0). A model for cases and controls is formed by pooling high risk cells into one group and low-risk cells into another group.
- Step 5: Select the best model based on classification error.
- Step 6: Evaluate the predictive ability of the best model in the test data set.

Multifactor Dimensionality Reduction



Results

Table 1. Baseline Characteristics of NNRTI Hepatotoxicity Subjects

Characteristic	Cases (n=20)	Controls (n=50)	p-value
Mean age years (SD)	39.01(7.44)	39.31(7.31)	0.343
Sex, n (%)			
Female	18 (90)	36 (72)	0.127
Male	2 (10)	14 (28)	
Race, n (%)			
Black	4 (20)	8 (16)	0.732
Caucasian	16 (80)	42 (84)	
HIV-1 RNA baseline (log10 mean(SD))	4.85 (0.54)	4.63 (0.86)	0.845
CD4 baseline median (IQR)	140 (289.5 - 60)	228 (312 - 128)	0.195
Albumin baseline median (IQR)	4.25 (4.43 - 3.75)	4.10(4.5 - 3.7)	0.945
BMI baseline mean (SD)	24.75 (2.69)	25.14 (6.75)	0.508
ALT prior >50. n (%)	11 (55)	10 (20)	0.007

Results

Table 2. Fisher's Exact Test Results

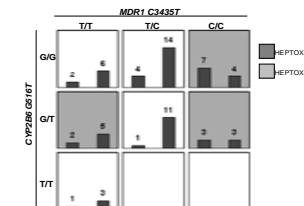
	Sample Size	OR	95% CI	p-value
<i>MDR1</i> C3435T	70	0.508	[0.232 - 1.115]	0.035
<i>CYP2B6</i> G516T	66	1.826	[0.834 - 3.958]	0.751
<i>CYP2B6</i> C1459T	67	3.228	[0.800 - 12.929]	0.446
<i>CYP3A4</i> A-392G	67	0.949	[0.412 - 2.187]	1.000
Hepatitis C Ab+	70	2.05	[0.56 - 7.43]	0.304
Hepatitis B S Ag+	64	11.73	[1.21 - 113.38]	0.024
PI with NNRTI	70	2.67	[0.92 - 7.73]	0.108
Alcohol*	69			0.310
Cocaine*	69			0.069

* During therapy

Multifactor Dimensionality Reduction

Number of loci	Locus	Cross validation consistency	Prediction Error
1	<i>CYP2B6</i> G516T	8	34.29
2	<i>CYP2B6</i> G516T, <i>MDR1</i> C3435T	6	31.43*
3	<i>CYP2B6</i> G516T, <i>CYP2B6</i> C1459T, <i>MDR1</i> C3435T	6	32.24
4	<i>CYP2B6</i> G516T, <i>CYP2B6</i> C1459T, <i>CYP3A4</i> A-392G, <i>MDR1</i> C3435T	10	36.21

Multifactor Dimensionality Reduction



Conclusions

- Cases, controls matched on age, race, NNRTI, HCV
- Also no difference in CD4, VL, sex, ETOH, PI
- Most hepatotoxicity occurred in women (NVP, EFV)
- Median CD4 < 250 in both cases and controls
- Based on single locus analysis, *MDR1* C3435T was associated with decreased hepatotoxicity risk (OR=0.508 [95%CI 0.232 - 1.115]; P=0.035)
- By Multifactor Dimensionality Reduction analysis, a two-locus interaction between *CYP2B6* G516T and *MDR1* C3435T was detected

Conclusions

- This 2-locus interaction predicted hepatotoxicity risk with approximately 70% accuracy (p<0.001)
- Multifactor Dimensionality Reduction may improve the predictive accuracy of polymorphisms
- It will be important to confirm the association of *MDR1* with hepatotoxicity risk in larger cohorts, which would allow for multivariate analysis