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Associations between *HLA-DRB1*0102*, *HLA-B*5801* and Hepatotoxicity during Initiation of Nevirapine-Containing Regimens in South Africa

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Introduction

A three-drug regimen comprising nevirapine plus two nucleoside reverse transcriptase inhibitors is frequently prescribed for HIV-1 infection worldwide. Unfortunately, drug hypersensitivity which can include severe hepatotoxicity during the initial weeks of therapy with nevirapine, particularly when initiated in women with >250 CD4+ T cells/mm³, have prompted changes in prescribing guidelines. Although isolated non-severe skin rash associated with nevirapine may be managed conservatively, the major treatment-limiting side effect of nevirapine is a drug hypersensitivity syndrome comprising fever, skin rash and/or hepatitis. This affects as many as 5% of those starting the drug.

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Potential conflicts of interest:

Elizabeth Phillips has received honoraria from Janssen-Cilag, Merck, Pfizer and ViiV Australia. Michael M. Lederman has served as consultant to Triangle Pharmaceuticals, Merck and ViiV. Franck Rousseau was an employee of Gilead at the time the study was conducted. Simon Mallal has received honoraria from ViiV and Merck.

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Various HLA class I and II alleles have been associated with nevirapine rash and/or hepatitis across different populations [1–6]. A population based study from Western Australia first identified an association between *HLA-DRB1*0101* and nevirapine-associated hepatitis among patients with ≥25% CD4+ T cells [1]. This work was complemented by *ex vivo* studies suggesting a CD4 T cell-dependent process. Associations were subsequently identified between various HLA class I alleles such as *HLA-B*1402* and *HLA-Cw8* and nevirapine hypersensitivity in Sardinian [2] and Japanese [3] populations. A case control study in a Thai population identified an association between *HLA-B*3505* and nevirapine-associated rash or hypersensitivity [4]. Further analyses from Western Australia confirmed the original association between *HLA-DRB1*0101* and ≥25% CD4 T cells, and identified an association between *HLA-B*3501* and nevirapine-associated rash, suggesting that phenotype was particularly important for defining specific HLA associations [5]. Only in the presence of hepatitis was *HLA-DRB1*0101* associated with rash, while *HLA-B*3501* was associated with rash regardless of hepatitis [5]. A retrospective analysis involving 76 sites in 11 countries (none in Africa) enrolled 276 cases who had experienced severe rash and/or hepatitis within 8 weeks of nevirapine initiation, and 587 nevirapine-tolerant controls [6]. Based on two digit HLA typing, that study associated *HLA-DRB1*01* with hepatitis in whites, both *HLA-B*35* and *HLA-Cw*04* with rash in Asians, and *HLA-Cw*04* alone with rash in Whites and Blacks. Furthermore, *CYP2B6* slow metabolizer genotype was associated with rash but not hepatotoxicity.

The above studies suggest that diverse genetic, immunological and drug metabolism pathways contribute to nevirapine hypersensitivity syndromes, and that associations may differ by specific toxicity phenotype and population. Data from Africa, where HIV prevalence and nevirapine use is high have been scant. Herein we used data and specimens previously collected during a clinical trial in South Africa to characterize relationships between HLA I and II alleles and nevirapine hepatotoxicity.

Materials and Methods

We analyzed data and specimens from participants who initiated nevirapine during Gilead protocol FTC-302, a cohort previously studied for associations with *CYP2B6* and *ABCB1* polymorphisms [7]. This randomized, double-blind trial enrolled antiretroviral naïve participants in South Africa in 1999 and 2000 to compare efficacy and safety of emtricitabine and lamivudine [8]. Eligible participants had >5,000 plasma HIV-1 RNA copies/mL and ≥200 CD4 cells/mm³. Participants were stratified to receive either nevirapine (200 mg once daily for two weeks, then 200 mg twice daily) or efavirenz (600 mg once daily). Because of limited data regarding nevirapine efficacy in participants with plasma HIV-1 RNA >100,000 copies/mL, participants with ≥100,000 copies/mL plasma HIV-1 RNA copies/mL were stratified to receive nevirapine whereas those with higher viral loads received efavirenz. All participants were also randomized 1:1 to receive emtricitabine (200 mg once daily) or lamivudine (150 mg twice daily), and all received stavudine (30 or 40 mg twice daily depending on weight). Only nevirapine recipients were included in the present analysis. Hepatotoxicity was defined as treatment-emergent grade 3 (5 to 10 times upper limit of normal [ULN]) or grade 4 (greater than 10 times ULN) elevation in serum aminotransferase (alanine aminotransferase [ALT]) or aspartate aminotransferase [AST]), with or without associated symptoms. To preserve anonymity the study sponsor matched each case with two controls on: race, Black versus other; sex; age; baseline CD4; and baseline HIV-1 RNA. Fever and rash were not exclusionary. Of all patients with liver toxicity on nevirapine, 45 had Grade 3/4 toxicity within 4 weeks of starting nevirapine, 1 had toxicity between 4 and 12 weeks, and 11 had toxicity beyond 12 weeks. This study complied with the Helsinki Declaration. Institutional review boards at Vanderbilt

University, Duke University, University of Witwatersrand and Murdoch University, approved this use of DNA.

Cryopreserved peripheral blood mononuclear cells (PBMCs) underwent DNA extraction with the Agencourt Genfind v2 DNA purification system (Beckman Coulter). High resolution HLA A B C and DR typing was done with standard sequence based typing at the Institute for Immunology & Infectious Diseases, Murdoch University [9].

Results

Among 385 nevirapine recipients on FTC-302, 66 (17%) experienced hepatotoxicity, which occurred within the first 12 weeks of therapy in 80% [8]. Of these 66 cases, 63 had PBMCs available of which 57 (87%) had adequate extracted DNA available for analysis. A total of 126 controls were identified, of whom 111 (88%) had specimens with adequate DNA for analysis following amplification. Baseline characteristics of cases and controls were similar. Mean age was 32 ± 7.0 years ($\pm$ SD), 113 (67%) were female, 120 (71%) were Black, 8 (14%) were Caucasian, 19 (11%) were Coloured (mixed race), 3 (2%) were Asian, and 1 (1%) were other, median baseline CD4 T cells were 381 (302 – 486) cells/mm³ (IQR), and mean baseline HIV-1 RNA was 4.3 ± 0.55 log₁₀ copies/mL- mean ($\pm$ SD).

By univariate analysis, HLA class I and II types associated with increased likelihood of hepatotoxicity included *B*0705*, *B*1801*, *B*5801*, *C*0302*, *C*1203* and *DRB1*0102* (Table 1, *top*). Conversely, HLA types associated with decreased likelihood of hepatotoxicity included *A*6801*, *B*1510*, *C*0210*, *C*0304* and *C*0602* (Table 1, *top*). By multivariable analysis, only *B*5801* and *DRB1*0102* were independently associated with increased likelihood of hepatotoxicity, while *B*1510*, *B*5802* and *C*0210* were independently associated with decreased likelihood of hepatotoxicity included (Table 1, *bottom*). There was no association between the nevirapine slow metabolizing genotype CYP2B6 516 G>T and hepatotoxicity and there was also no association between this drug metabolizing genotype and HLA class I or II alleles in either model.

Discussion

Various associations have been reported between HLA types and nevirapine-associated adverse events, but data from Africa have been lacking. We provide the first report, in a predominantly Black South African cohort that both HLA-B*5801 and HLA-DRB1*0102 were independently associated with increased likelihood of hepatotoxicity during combination antiretroviral therapy that included nevirapine. Hepatotoxicity in FTC-302, and therefore in the present analysis, was almost certainly due to nevirapine, since grade 3 or 4 hepatic transaminase elevations affected 17% of nevirapine recipients but 0 of 83 efavirenz recipients [8].

The matched case-control design used in this study minimizes effects of potential confounders, and may increase power to identify genetic associations. Although this prevented us from exploring associations between the matching variables such as CD4+ T cell count/percentage and hepatotoxicity, extensive analyses of possible non-genetic predictors of hepatotoxicity have already been reported for FTC-302. Female sex, low body mass index, serum albumin, plasma HIV RNA, high mean corpuscular volume, LDH and AST levels were significantly and independently associated with subsequent risk of hepatotoxicity in nevirapine recipients, whereas hepatitis B or C co-infection, other potentially hepatotoxic drugs, and traditional medication use were not. The well established association of nevirapine-induced liver toxicity with higher CD4+ T cell counts was not

apparent in FTC-302 likely because few nevirapine recipients had lower CD4 cell counts [8].

The translation of *HLA-B*5701* screening into routine HIV clinical care has markedly reduced the occurrence of abacavir hypersensitivity reactions and improved patient safety. Reliable genetic predictors of nevirapine toxicity might allow HIV therapy that includes nevirapine to be initiated at higher CD4+ T cell counts. In addition, the association of HLA class I alleles such as *HLA-B*35* in multiple studies and *HLA-B*5801* in this study is strong evidence that CD8+ and not just CD4+ T cells contribute to the immunopathogenesis of nevirapine hypersensitivity. This is further supported by studies in nevirapine hypersensitive patients showing nevirapine specific HLA class I restricted CD8+ cells and significant abrogation of nevirapine specific ex vivo responses with depletion of CD8+ T cells [5]. *HLA-B*5801* has also been associated with allopurinol, a drug used to treat gout, that causes Stevens-Johnson syndrome/toxic epidermal necrolysis and hypersensitivity in Han Chinese populations [10]. The association between *HLA-DRB1*0102* and hepatotoxicity in this South African nevirapine-treated population is intriguing given that *HLA-B*DRB1*0102* only differs from *HLA-DRB1*0101* by two amino acids at position 85 (val→ala) and 86 (gly→val), and *HLA-DRB1*01* has been reproducibly associated with nevirapine rash-associated hepatitis in white populations[1,5,6]. Class I alleles which have previously been associated with nevirapine hypersensitivity such as *HLA-B*35*, *B*1402* and *Cw8* were not prevalent in this population and therefore those associations could not be adequately re-examined here.

Conclusions

Recent studies have highlighted varying HLA associations with nevirapine hypersensitivity, particularly in White and Southeast Asian populations. Our results for the first time support the diversity of HLA class I and II alleles associated with nevirapine hepatotoxicity in particular Black African populations, suggest some HLA alleles may indeed be protective against the development of nevirapine hepatotoxicity, and underscore the need for HLA studies of drug hypersensitivity in populations where different HLA alleles may be prevalent.

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Table 1Univariate and Multivariate Associations between HLA Types and Nevirapine-associated Hepatotoxicity¹⁻²

HLA Allele	Cases n/N (%)	Controls n/N (%)	P-value (Relative Risk)
<i>Univariate analysis</i>			
<i>A*6801</i>	0/53 (0%)	8/103 (7.8%)	0.017 (NA)
<i>B*0705</i>	3/53 (5.7%)	1/106 (0.9%)	0.010 (NA)
<i>B*1510</i>	4/53 (7.5%)	20/106 (18.9%)	0.042 (0.31)
<i>B*1801</i>	4/53 (7.5%)	2/106 (1.9%)	0.096 (4.0)
<i>B*3501</i>	1/53 (1.9%)	4/106 (3.8%)	0.47 (0.44)
<i>B*3505</i>	1/53 (1.9%)	1/106 (0.9%)	0.81 (1.4)
<i>B*4101</i>	0/53 (0%)	4/106 (3.8%)	0.072 (NA)
<i>B*5801</i>	12/53 (22.6%)	9/106 (8.5%)	0.018 (3.2)
<i>B*5802</i>	6/53 (11.3%)	25/106 (23.6%)	0.057 (0.42)
<i>C*0210</i>	2/55 (3.6%)	19/106 (17.9%)	0.0012 (0.09)
<i>C*0302</i>	6/55 (10.9%)	3/106 (2.8%)	0.041 (4.0)
<i>C*0304</i>	4/55 (7.3%)	19/106 (17.9%)	0.047 (0.32)
<i>C*0602</i>	12/55 (21.8%)	39/106 (36.8%)	0.042 (0.45)
<i>C*1203</i>	8/55 (14.5%)	4/106 (3.8%)	0.017 (4.6)
<i>DRB1* 0101</i>	0/54 (0%)	3/103 (2.9%)	0.14 (NA)
<i>DRB1* 0102</i>	8/54 (14.8%)	4/103 (3.9%)	0.022 (4.3)
<i>DRB1* 0301</i>	4/54 (7.4%)	15/103 (14.6%)	0.086 (0.36)
<i>DRB1* 1401</i>	0/54 (0%)	4/103 (3.9%)	0.072 (NA)
<i>Multivariable analysis (increased risk)</i>			
<i>B*5801</i>	12/51 (23.5%)	9/102 (8.8%)	0.033 (3.81)
<i>DRB1* 0102</i>	8/51 (15.7%)	4/102 (3.9%)	0.044 (4.72)
<i>(decreased risk)</i>			
<i>B*1510</i>	4/51 (7.8%)	20/102 (19.6%)	0.010 (0.11)
<i>B*5802</i>	5/51 (9.8%)	24/102 (23.5%)	0.005 (0.12)
<i>C*0210</i>	2/51 (3.9%)	17/102 (16.7%)	0.006 (0.04)

¹ Analyses were carried out in TIBCO Spotfire S+ ver 8.1 (TIBCO Software, Inc, Palo Alto, Calif) using conditional logistic regression to accommodate the matching of cases and controls.

² P-values associated with alleles previously associated with nevirapine hypersensitivity or those with p-values below or near 0.05 without considering multiple comparisons.