



Published in final edited form as:

J Acquir Immune Defic Syndr. 2010 July 1; 54(3): 285–289. doi:10.1097/QAI.0b013e3181bf648a.

CYP2C19 Genetic Variants Affect Nelfinavir Pharmacokinetics and Virologic Response in HIV-1–Infected Children Receiving Highly Active Antiretroviral Therapy

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Abstract

Background—The objective of this research was to identify the impact of genetic variants of P-glycoprotein (ABCB1) and cytochrome P450 (CYP) on nelfinavir pharmacokinetics and response to highly active antiretroviral therapy (HAART) in HIV-1–infected children.

Methods—HIV-1–infected children (n = 152) from Pediatric AIDS Clinical Trial Group 366 or 377 receiving nelfinavir as a component of HAART were evaluated. Genomic DNA was assayed for ABCB1 and CYP genetic variants using real-time polymerase chain reaction. Nelfinavir oral clearance (CL/F), M8 to nelfinavir ratios, CD4⁺ T cells, and HIV-1-RNA were measured during HAART.

Results—Nelfinavir CL/F and M8 to nelfinavir ratios were significantly associated with the CYP2C19-G681A genotypes ($P < 0.001$). Furthermore, the CYP2C19-G681A genotype was related to virologic responses at week 24 ($P = 0.01$). A multivariate analysis demonstrated that age ($P = 0.03$), concomitant protease inhibitor use ($P < 0.001$), and the CYP2C19-G681A genotype ($P < 0.001$) remained significant covariates associated with nelfinavir CL/F.

Conclusions—CYP2C19 genotypes altered nelfinavir pharmacokinetics and the virologic response to HAART in HIV-1–infected children. These findings suggest that CYP2C19 genotypes are important determinants of nelfinavir pharmacokinetics and virologic response in HIV-1–infected children.

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Presented in part at the: 15th Conference on Retroviruses and Opportunistic Infections, February 2008, Boston, MA. Poster #572.

Keywords

ABCB1; CYP2C19; children; nelfinavir; virologic response

Introduction

Protease inhibitors (PIs), which have been used widely as a component of highly active antiretroviral therapy (HAART) in children and adults are known to be a substrate of P-glycoprotein¹ and metabolized by hepatic cytochrome P450 (CYP), mainly by CYP3A4.² In particular, nelfinavir (NFV) is metabolized into the metabolite hydroxyl-tert-butylamide (M8) by the CYP2C19 enzyme, and subsequently NFV and M8 are metabolized by CYP3A4.³ Several pharmacogenetic studies have shown that single nucleotide polymorphisms (SNPs) in adenosine triphosphate-binding cassette, subfamily B, member 1 *ABCB1* (previously called multidrug resistance 1, *MDR1*)⁴ and *CYP*⁵ can influence the activity and bioavailability of NFV.

We previously reported that genetic variants in *ABCB1* gene encoding for P-glycoprotein was responsible for variability in NFV pharmacokinetics (PKs) and virologic responses to HAART in children.⁶ However, the study was limited because of the small number of subjects. In addition, our earlier results were in contrast to a study previously reported in adults.⁴ Therefore, to expand the number of subjects, we examined children who received NFV as a component of HAART from 2 pediatric studies, Pediatric AIDS Clinical Trial Group (PACTG) 366⁷ and 377,⁸ to investigate the association between NFV PK and SNPs in *ABCB1* and *CYP* and virologic and immunologic responses.

Materials and Methods

Subjects

This was a retrospective study investigating 152 children who received NFV as a component of HAART regimens although participating in PACTG 366 (n = 75, 49%) and PACTG 377 (n = 77, 51%) (Table 1). Informed consent was obtained from study participants. This study followed the human experimentation guidelines of the US Department of Health and Human Services and the University of California, San Diego review board.

NFV PKs

Among the 152 patients, 106 children (70%) had intensive PK collected over 8 hours at week 4 of treatment and 46 subjects (30%) had sparse PK during HAART. These results and detailed methods for the measurement of NFV and calculation of PK parameters have been previously reported from subjects participating in PACTG 377.^{8,9} The M8 to NFV ratios were calculated based on each M8 and NFV value and averaged in subjects with the intensive PK data. When the M8 level was <50 ng/mL, such numbers were imputed to 50 ng/mL.⁵

Measurement of Plasma HIV-1 RNA and CD4⁺ T Cells

Plasma HIV-1 RNA was quantified using the Roche Amplicor HIV-1 Monitor assay (Roche Molecular Systems, Alameda, CA) with a detection limit of 400 copies per milliliter. The numbers and percentages of CD4⁺ T cells were determined in PACTG certified laboratories by flow cytometry.

Amplification and Detection of Polymorphisms in *ABCB1* and *CYP* Genes by Real-Time Polymerase Chain Reaction

Previously developed fluorescence assays and detection methods were used for analyzing the *ABCB1*-3435C>T (rs1045642) and *CYP3A4*-392A>G (rs2740574).⁶ For the *ABCB1*-2677G>T (rs2032582),¹⁰ *CYP2C19C**2-681G>A (rs4244285), and *CYP2C19**3-636G>A (rs28399504),¹¹ previously reported assays were used. In addition, novel fluorescent assays were developed to detect the *ABCB1*-1236C>T (rs1128503) and *ABCB1*-1199G>A (rs2229109) genotypes. The information regarding the sequences of custom designed primers and probes and polymerase chain reaction conditions are available upon request.

Statistical Analysis

Statistical analyses were performed using the SPSS 13.0 software (Chicago, IL). Comparisons among ordered 3 genotypes at each categorical group were performed using the Jonckheere-Terpstra (rank-based trend) test for continuous outcomes and Cochran-Armitage trend test for binomial outcomes. A multivariate analysis for NFV CL/F was performed to evaluate the contribution of covariates. The χ^2 and Fisher exact tests were used to make pair-wise comparisons. All *P* values calculated were 2 sided and *P* value <0.05 was considered to be significant.

Results

Frequencies of *ABCB1* and *CYP* Gene Polymorphisms

The *ABCB1*-3435-T allelic frequency was 0.35 in the whole cohort; however, the frequencies were less common in black, non-Hispanic (*P* = 0.006). Similarly, there were significant differences in the frequencies of *ABCB1*-G2677T (*P* < 0.001) and *ABCB1*-C1236T genotypes (*P* < 0.001) in black, non-Hispanic, compared with others. The *CYP2C19*-681-A allelic frequency was 0.19 in whole cohort, and the frequencies were similar among race/ethnicity (*P* = 0.35) (Table 1). All patients had the *CYP2C19*-636-G/G genotype.

The *ABCB1*-3435C> T Genotype is Associated With NFV CL/F

NFV CL/F differed significantly among the *ABCB1*-3435C>T genotype (*P* < 0.001); children with the *ABCB1*-3435-C/C genotypes had higher median NFV CL/F [47.2 L/h/m², interquartile range (IQR): 32.7–68.7 L·h⁻¹·m⁻²] compared with those with the C/T (36.1 L/h/m², IQR: 28.1–56.7 L·h⁻¹·m⁻²) and the T/T genotype (35.4 L/h/m², IQR: 17.8–61.3 L·h⁻¹·m⁻²). NFV CL/F did not differ among subjects with the other *ABCB1* genotype (*P* > 0.17). There was no difference in the association when examined by each race/ethnic group (*P* > 0.11).

The *CYP2C19*-681G>A Polymorphism is Associated With NFV CL/F

NFV CL/F differed significantly among the *CYP2C19*-681G>A genotypes (*P* < 0.001). When the data were analyzed in each race/ethnicity (Fig. 1), a significant difference in NFV CL/F was observed in black, non-Hispanics (*P* < 0.001). The same trends were also observed for Hispanics (*P* = 0.12) and white, non-Hispanics (*P* = 0.25), but the differences did not achieve the level of significance.

Association Between the *CYP2C19*-681G>A Genotype and the M8 to NFV Ratio

Overall, the median M8 to NFV ratio was associated with the *CYP2C19*-681G>A genotype (*P* < 0.001); the ratio was 0.45 (IQR: 0.22–0.96) in those with the -G/G genotype compared with 0.26 (IQR: 0.13–0.47) in -G/A or 0.02 (IQR: 0.01–0.08) for the -A/A genotype. The

association between the *CYP2C9*-681G>A genotype was particularly strong for the black, non-Hispanic group ($P < 0.001$), but not for the Hispanic ($P = 0.56$), and white, non-Hispanic ($P = 0.30$) groups. No other genotypes were associated with the M8 to NFV ratio ($P = 0.29$ – 0.87).

Virologic and Immunologic Responses During HAART in Children With *ABCB1* and *CYP* Genotypes

The percentages in children who reached plasma HIV RNA <400 copies per milliliter at week 12 did not differ by the *CYP2C19*-681G>A genotypes ($P = 0.14$ – 1.00). However, at week 24, the percentage of subjects among the *CYP2C19*-681G>A genotype who reached plasma HIV RNA <400 copies per milliliter differed significantly ($P = 0.01$): 46% of subjects with the *CYP2C19*-681-G/G genotype achieved virologic suppression compared with 69% of those with the -G/A genotype, and 63% of those with the -A/A genotype. When examined by race/ethnicity, these differences were observed for the black, non-Hispanic group ($P = 0.02$) and the white, non-Hispanic group ($P = 0.03$), but not for Hispanics ($P = 0.84$). No differences were observed when the data were analyzed with the *ABCB1*-3435C>T genotype ($P = 0.06$) or the *CYP3A4*-392A>G genotype at week 24 ($P = 0.26$). Regarding immunologic recovery, changes in CD4⁺ T-cell percentage from baseline to weeks 12 and 24 were not different among the 3 genotypes in *CYP2C19*-681G>A ($P = 0.50$, $P = 0.44$, respectively) or *ABCB1*-3435C>T ($P = 0.08$, $P = 0.21$, respectively).

Other Factors Contributing to NFV CL/F Concomitant Antiretrovirals

Because nevirapine induces hepatic CYP3A and decreases the levels of PIs¹² and ritonavir acts as a potent PK enhancer for CYP substrates,¹³ we evaluated the association between NFV CL/F and concomitant use of nevirapine or ritonavir. NFV CL/F was not different between subjects who received nevirapine and those who did not receive nevirapine ($P = 0.70$). In contrast, ritonavir use decreased NFV CL/F significantly ($P = 0.002$); the median NFV CL/F in patients who received ritonavir (35.8 L/h/m², IQR: 24.7–47.5 L·h⁻¹·m⁻²) was lower compared with those who did not receive ritonavir (47.4 L/h/m², IQR: 32.5–70.6 L·h⁻¹·m⁻²).

Association of Race/Ethnicity on NFV CL/F and Clinical Outcomes

Because race/ethnicity is an important determinant of these SNPs, we also analyzed the data based on their race/ethnicity. Black, non-Hispanics (43.4 L·h⁻¹·m⁻², IQR: 33.1–66.6 L·h⁻¹·m⁻²) and Hispanics (45.2 L·h⁻¹·m⁻², IQR: 26.2–65.2 L·h⁻¹·m⁻²) had higher median NFV CL/F compared with white, non-Hispanics (31.7 L·h⁻¹·m⁻², IQR: 27.3–53.3 L·h⁻¹·m⁻²), but it did not reach a statistical significance ($P = 0.09$). M8 to NFV ratio was not associated with race/ethnicity ($P = 0.67$). Furthermore, clinical outcomes including percentages in children who reached plasma HIV RNA <400 copies per milliliter at week 12 ($P = 0.54$) or changes in CD4⁺ T-cell percentage from baseline to weeks 12 ($P = 0.89$) was not associated with race/ethnicity.

A Multivariate Analysis for Predicting NFV CL/F

A multivariate analysis showed that the *CYP2C19*-681G>A variants ($P < 0.001$), concomitant use of ritonavir ($P < 0.001$), and age ($P = 0.03$) were independently associated with NFV CL/F. However, the *ABCB1*-3435 variants ($P = 0.61$), *CYP3A4*-392 homozygous variants ($P = 0.42$), and race/ethnicity (black, non-Hispanics) ($P = 0.07$) were no longer statistically significant. Thus, the *CYP2C19*-681G>A genotype remains an important pharmacogenetic determinant of NFV CL/F even after controlling for other factors.

Discussion

The data presented here demonstrate that *CYP2C19*-681G>A variants exert the greatest impact on NFV PK and virologic response. Controlling for various factors, only *CYP2C19*-681G>A genotype and concomitant PI usage continued to demonstrate a highly significant association with NFV CL/F.

Hepatic *CYP2C19* is the critical enzyme responsible for conversion of NFV to its M8 metabolite.¹⁴ Alteration in *CYP2C19*-681G>A in exon 5, which creates an aberrant splice site resulting in a truncated nonfunctional protein¹⁵ is the SNP identified in *CYP2C19* most often associated with different clinical responses to pharmacologic agents for treating diseases (eg, treatment of peptic ulcer disease using proton pump inhibitors).¹⁶ Previous reports have described the impact of this genotype on the NFV to M8 ratio in HIV-1–infected adult populations.^{5,17,18} Notably, Haas et al⁵ reported a trend toward decreased virologic failure associated with the *CYP2C19*-681G>A genotype. Our current data in children is in agreement with the Haas study and provide further support for an important role of *CYP2C19*-681G>A variants in NFV PK and virologic response. We cannot rule out that other *CYP2C19* SNPs might also alter the PK of NFV or other PIs.¹⁹ In addition, we only investigated 7 SNPs which have been reported to be related to NFV PK. Furthermore, when we analyzed the data by each race/ethnicity, significant differences in NFV PK were only observed in black, non-Hispanics and virologic response in black, non-Hispanics and white, non-Hispanics. These apparent differences are likely related, in part, to the lower number of study participants in the white, non-Hispanic and Hispanic cohorts.

NFV was used extensively in years past but is now rarely used in clinical practice for children in developed countries for a few reasons; NFV has been replaced by more potent PIs (eg, lopinavir/ritonavir) with better PK profile and fewer incidences of adverse effects (eg, diarrhea) and recent manufacture problem with a contamination of ethyl methylate.²⁰ However, NFV continues to be used in developing countries. The information learned in this current study may be helpful to improve the clinical outcomes of children who receive NFV.

In conclusion, the *CYP2C19*-681G>A, age, and concomitant ritonavir are significantly associated with NFV PK in HIV-1–infected children. In addition, favorable virologic response was observed in children with the *CYP2C19*-681G>A variants associated with lower oral NFV CL/F. These findings suggest that *CYP2C19*-681G>A is the most important pharmacogenetic determinant for NFV PK and virologic responses in children who receive HAART-containing NFV.

Acknowledgments

Supported by the Pediatric AIDS Clinical Trials Group/International Maternal, Perinatal, Adolescent AIDS Clinical Trials Group, by Grants from the National Institute of Allergy and Infectious Diseases [(U01A141089 and 5K23AI-56931 to AS, AI-41089, AI-39004, AI-27563, AI-33835, AI-41110); AI-36214 (Virology Core University of California, San Diego Center for AIDS Research), AI-32921, AI-68632 and AI-68616] and by Bristol-Myers Squibb.

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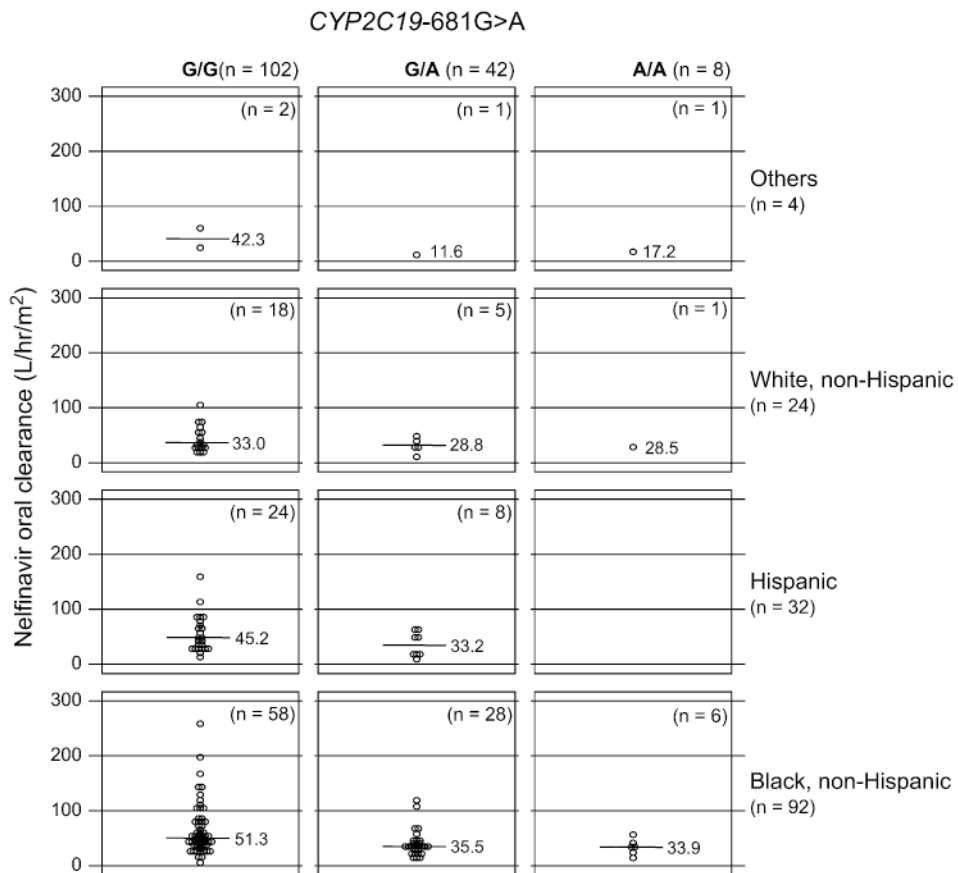


Figure 1. Oral clearance rate (CL/F, L/h/m²) for nelfinavir in children with the *CYP2C19*-681G>A genotypes in each race/ethnicity. Each circle represents nelfinavir CL/F in each subject with the *CYP2C19*-681-G/G (left), -G/A (heterozygous, middle), and -A/A (homozygous, right) in each race/ethnicity. The lines in the middle represent the median of CL/F for NFV.

Table 1
Baseline Characteristics of the 152 Children in PACTG 366 and 377 With the CYP2C19-G681A Genotypes

	All Subjects*		PACTG 366		PACTG 377		CYP2C19-G681A		
	n = 152 (%)	n = 75 (49)	n = 77 (51)	P	G/G n = 102 (%)	G/A n = 42 (%)	A/A n = 8 (%)	P	
Sex, n (%)									
Male	79 (52)	46 (61)	33 (43)	0.01	53 (67)	22 (28)	4 (5)	0.99	
Female	73 (48)	29 (39)	44 (57)		49 (67)	20 (27)	4 (6)		
Race/ethnicity									
Black, non-Hispanic	92 (61)	45 (60)	47 (61)	0.73	58 (63)	28 (30)	6 (7)	0.35	
Hispanic	32 (21)	15 (20)	17 (22)		24 (75)	8 (25)	0 (0)		
White, non-Hispanic	24 (16)	12 (16)	12 (16)		18 (75)	5 (21)	1 (4)		
Others	4 (3)	3 (4)	1 (1)		2 (50)	1 (25.0)	1 (25.0)		
Age (yrs) [median, (IQR)]	7.1 (3.8-9.6)	6.9 (3.2-10.6)	6.8 (4.4-9.2)	0.42	7.1 (3.5-10.1)	7.1 (3.9-9.4)	7.9 (5.9-10.7)	0.82	
Concomitant PI or NNRTI									
No	23 (15)	0	23 (30)		12 (52)	9 (39)	2 (9)	0.63	
Ritonavir	40 (26)	40 (53)	0		29 (73)	9 (23)	2 (5)		
Nevirapine	60 (40)	6 (8)	54 (70)		43 (72)	14 (23)	3 (5)		
Ritonavir + nevirapine	29 (19)	29 (39)	0		18 (62)	10 (35)	1 (3)		
Baseline CD4+ (%) median, (IQR)	24 (16-32)	19 (10-28)	27 (21-35)	<0.001	23 (16-32)	25 (19-31)	22 (6-40)	0.85	
Mean HIV-1 RNA log ₁₀ copies/mL (SD)	4.56 (0.68)	4.71 (0.61)	4.41 (0.72)	0.003	4.54 (0.70)	4.62 (0.64)	4.44 (0.74)	0.65	

* The subjects were selected from the whole study populations if they satisfied the following criteria: (1) received NFV as a component of HAART for >24 weeks with reported excellent compliance to their treatment regimen; (2) virologic and immunologic data were available at baseline, weeks 12 and 24; and (3) PK data for NFV were available at week 4.

NNRTI, nonnucleoside reverse transcriptase inhibitor.