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Prevalence and risk factors associated with resistance-associated mutations to etravirine in a cohort of perinatally HIV-infected children

German A. Contreras[1,2], Cynthia S. Bell[3], Gabriela P. Del Bianco[1], Norma Pérez[1], Matthew T. Kleinosky[1], James R. Murphy[1] and Gloria P. Heresi[1]*

[1]*Division of Paediatric Infectious Diseases, Department of Paediatrics, Medical School, UTHealth, 6431 Fannin Street MSB 3.020, Houston, TX 77030, USA;* [2]*Molecular Genetics and Antimicrobial Resistance Unit, Bosque University, Bogotá, Colombia;* [3]*Division of Paediatric Nephrology and Hypertension, Department of Paediatrics, Medical School, UTHealth, 6431 Fannin Street MSB 3.121, Houston, TX 77030, USA*

*Corresponding author. E-mail: gloria.p.heresi@uth.tmc.edu

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Background: Etravirine is a second-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) with reduced cross-resistance to first-generation NNRTIs. Because many perinatally HIV-infected patients have been treated with first-generation NNRTIs, they may have acquired resistance-associated mutations to etravirine (RAMe).

Methods: We determined for the interval 1998–2009 the prevalence and factors associated with the presence of RAMe.

Results: Twenty-three of 66 (34.8%) children had RAMe; the most common were 181C (19.6%), 190A (7.5%), 98G (6%), 106I (4.5%), 179D (4.5%), 100I (3%), 181I (1.5%), 138A (1.5%) and 179T (1.5%). Eleven children with RAMe (17%) had a mutation score between 2.5 and 3.5 and 1 (1.5%) a score ≥4, indicating an intermediate and reduced response to etravirine. For each 1% increase in CD4% there is a 7% decrease in the odds of RAMe (OR 0.93; 95% CI 0.88–0.97; $P<0.01$). History of nevirapine use (OR 8.95; 95% CI 2.31–34.73; $P<0.01$) and Hispanic ethnicity (OR 4.76; 95% CI 1.03–21.87; $P=0.04$) are significantly associated with risk of RAMe.

Conclusions: RAMe are present and common among antiretroviral-experienced perinatally HIV-infected children without previous exposure to etravirine. This could limit the efficacy of etravirine-based regimens. In addition, our results underscore the importance of taking previous history of nevirapine into account for combined antiretroviral therapy regimens that contain etravirine.

Keywords: therapy adherence, cumulative HIV RNA, Hispanics

Introduction

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are an important component of combined antiretroviral therapy (cARV); however, resistance to first-generation NNRTIs emerges rapidly due to a low genetic barrier.[1] Etravirine is a second-generation, potent NNRTI, which, in contrast to first-generation NNRTIs, requires multiple mutations in order to compromise its antiviral activity. Several clinical trials demonstrated the efficacy and safety of etravirine for the management of treatment-experienced adult patients with documented resistance to efavirenz and nevirapine.[2] Furthermore, similar response has been shown among treatment-experienced infected children and adolescents aged 6 to <18 years.[3,4]

Seventeen mutations have been associated with etravirine resistance among drug-experienced adults.[5] Recently, a number of studies reported the emergence of resistance-associated mutations to etravirine (RAMe) among children in developing countries with a history of failing first-generation NNRTI-based regimens and no previous exposure to etravirine.[6,7] However, data on etravirine resistance in children from the USA are limited, especially for those with documented exposure to NNRTI-based regimens.[8] The aim of this study was to determine the prevalence and risk factors associated with the presence of RAMe in a cohort of drug-experienced perinatally HIV-infected children.

Methods

We present a retrospective cohort study comprising children and adolescents with perinatally acquired HIV who received routine care at UTHealth Houston between September 1998 and February 2009. All patients attending the clinic were reviewed for inclusion and those who had at least one resistance mutation (RM) test and clinical follow-up of ≥1 year were included in the analysis. The study was approved by the institutional review board of UTHealth. Written informed consent was obtained.

Prevalence of RAMe

We determined the prevalence of 17 RAMe in a cohort of children with perinatally acquired HIV from October 1998 to February 2009. A historical mutation profile was compiled using all available genotypes for each subject.[9] Positive RAMe was defined as the presence of etravirine-specific mutations at any genotype test during a subject's entire historical profile following the definition proposed by Vingerhoets *et al.*[5] Prevalence estimates were calculated as the number of children and adolescents with RAMe divided by the total number of children and adolescents with RM tests. We defined four birth cohorts (1980–90, 1991–95, 1996–99 and 2000–07) that correspond to changes in the availability of antiretroviral drugs.[8]

Therapy adherence

A medication adherence questionnaire used as part of the Ryan White Program was used to document adherence. The questionnaire was provided either by the physician or social worker and completed either by the parent or legal guardian. The questionnaire was available in both English and Spanish.

Factors associated with RAMe

A logistic regression model was used to determine predictors of RAMe. The analysis for each patient is anchored to the date of the first RAMe. Dummy variables were created for birth cohort, sex and race, where youngest birth cohort, female and non-Hispanic were selected as reference groups. Years of observation, CD4%, cumulative HIV RNA and age at the first RAMe detection were continuous variables. History of AIDS and exposure to nucleoside reverse transcriptase inhibitors (NRTIs), NNRTIs, protease inhibitors, monotherapy, dual therapy and cARV were evaluated as categorical variables. Cumulative HIV RNA was calculated as the sum of daily HIV RNA copies from the first measurement of viraemia until the date of the first RAMe detection or 28 February 2009, whichever came first, and is represented as the $\log_{10}$ of this sum. HIV RNA on days between measurements was estimated using the trapezoidal rule and undetectable HIV RNA was recorded as 400 copies/mL.[10] Variables with >10% missing data were excluded.

For primary analyses, we fitted univariate models to evaluate each potential predictor of RAMe. All variables with a P value <0.1 in the univariate analysis were included in a multivariate model. Statistical significance was set at the two-tailed 5% level ($P<0.05$). Collinearity was checked during the variable selection process and tested using rank sum or χ^2 tests.

Results

Of 97 potentially eligible patients, 66 (68%) had at least one RM test and 31 (32%) did not (16 transferred to another facility or left Houston, 7 had repeatedly undetectable viraemia and 8 had phenotype tests). The 31 patients excluded had a similar race distribution to the included patients ($P=0.617$) black 22 (71%), Hispanic 6 (19.4%) and white 3 (9.7%); 26 were followed for >1 year, where all had a history of virologic failure by the end of the follow-up.

Of the 66 included patients, 56 had virologic failure and 10 were antiretroviral drug naive. Thirty-four (51.5%) and 32 (48.5%) had at least three tests or less than three tests, respectively. Of the 66, 74.2% were black and 57.6% were female. The first RM test was conducted at a median 6.3 years of age (IQR 2.2–9.8) and the median observation interval was 10.1 years (IQR 6.6–14.4). The use of nevirapine and efavirenz in this cohort started at the beginning of 1997 and 1999, respectively, with efavirenz becoming the most frequently used NNRTI in 2005. However, <20% of children were receiving either drug by 2009. Of the 66, 28 (42.4%) had a history of exposure to NNRTIs, with nevirapine being the most common (36.4%) (Table 1). Of the 28, 20 had a history of failing NNRTI regimens (defined as the presence of a plasma HIV RNA ≥1000 copies/mL after 6 months on NNRTI therapy).

Table 1. Characteristics of perinatally HIV-infected children

Characteristic	RAMe	
	absent (43)	present (23)
Years of observation	9.46 (5.9–12.6)	13.77 (7.6–16.2)[a]
Age at first RM test (years)	5.75 (2.1–9.8)	7.39 (2.7–11.9)
Male	17 (40)	11 (48)
Race/ethnicity		
black	34 (79.1)	15 (65.2)
Hispanic (not black)	6 (14.0)	8 (34.8)
white	3 (7.0)	0 (0)
Birth cohort		
1980–90	5 (11.6)	3 (13.0)
1991–95	17 (39.5)	12 (52.2)
1996–99	11 (25.6)	4 (17.4)
2000–07	10 (23.3)	4 (17.4)
At the time of RAMe detection		
CD4%	34 (26–42)	22 (16–31)[a]
HIV RNA (copies/mL, $\log_{10}$)	3.2 (2.6–3.9)	3.6 (2.6–4.6)
cumulative HIV RNA (copies/mL, $\log_{10}$)[b]	7.9 (7.1–8.2)	7.9 (7.2–8.6)
History of		
AIDS	13 (30.2)	9 (39.1)
monotherapy	16 (37.2)	13 (56.5)
dual therapy	20 (46.5)	15 (65.2)
cARV	35 (81.4)	19 (82.6)
NRTI	42 (97.7)	21 (91.3)
protease inhibitor	35 (81.4)	19 (82.6)
NNRTI	10 (23.3)	18 (78.3)[a]
length of exposure (years)	4.9 (2.8–5.5)	2.6 (1.5–6.0)
adherence	6 (60)	9 (50)
efavirenz	8 (18.6)	6 (26.1)
birth cohort		
1980–90	2 (4.7)	2 (8.7)
1991–95	4 (9.3)	3 (13.0)
1996–99	2 (4.7)	1 (4.3)
2000–07	0	0
nevirapine	9 (20.9)	15 (65.2)[a]
birth cohort		
1980–90	2 (4.7)	2 (8.7)
1991–95	2 (4.7)	9 (39.1)
1996–99	3 (7.0)	1 (4.3)
2000–07	2 (4.7)	3 (13.0)
efavirenz and nevirapine	4 (9.3)	3 (13.0)

Data are presented as median (IQR) or number (%).
[a]$P<0.05$.
[b]Sum of measured interpolated daily viraemias from the first HIV RNA determination until the date of RAMe detection or 28 February 2009, whichever came first.

Prevalence of RAMe

Of the 66, 23 (34.8%) had at least one RAMe; the most common were 181C (19.6%), 190A (7.5%), 98G (6%), 106I (4.5%), 179D (4.5%), 100I (3%), 181I (1.5%), 138A (1.5%) and 179T (1.5%). Among the 10 antiretroviral drug-naive patients, 181C ($n=2$), 138A ($n=1$) and 98G ($n=1$) were the most common mutations detected. When the overall prevalence of RAMe was parsed by birth cohort [1980–90=3 (4.5%), 1991–95=12 (18.2%), 1996–99=4 (6.1%) and 2000–07=4 (6.1%)], we observed a notably higher proportion among patients born between 1991 and 1995, but the differences did not reach significance, most likely because of the small number of individuals in some cohorts. Overall, 11 of the patients with RAMe (17%) had a mutation score between 2.5 and 3.5 and 1 (1.5%) had a score ≥4, indicating an intermediate and reduced response to etravirine (Patients 1–7, 181C; Patient 8, 100I; Patient 9, 181I; Patient 10, 100I+179T; Patient 11, 181C+98G; and Patient 12, 181C+98G+190A).

Of the 23 patients with RAMe, 18 had a significantly higher exposure to NNRTIs (78.3%, $P=0.002$ by Fisher's exact test) compared with patients without RAMe (23.3%) (Table 1). Fourteen (77.8%) of the 18 patients had a history of NNRTI failure. Of the 18, 9 (50%) and 1 (5.6%) had an intermediate and reduced response to etravirine.

Predictors

Univariate analysis showed that previous history of use of NNRTIs, particularly nevirapine, was significantly associated with RAMe (Table 2). Similarly, Hispanics and patients with longer periods of observation had increased probabilities of developing RAMe. For each 1% increase in CD4% there is a 7% decrease in the odds of RAMe (OR 0.93; CI 0.88–0.97; $P<0.01$) (Table 2).

Due to the requirement in logistic regression that all predictors must be independent from each other, both NNRTIs and nevirapine

Table 2. Predictors of RAMe among perinatally HIV-infected children

	Univariate			Multivariate		
Variable	OR	95% CI	P^a	OR	95% CI	P^a
Years of observation	1.11	1.00–1.23	0.06			
Age at first RM test (years)	1.04	0.94–1.15	0.49			
Male	1.40	0.50–3.89	0.52			
Ethnicity						
non-Hispanic[b]	1.0	1.0				
Hispanic	3.29	0.97–11.10	0.06	4.76	1.03–21.87	0.04
Birth cohort						
1980–90	1.50	0.24–9.46	0.67			
1991–95	1.76	0.45–6.98	0.42			
1996–99	0.91	0.18–4.64	0.91			
2000–07	1.0	1.0				
CD4%	0.93	0.88–0.97	<0.01	0.94	0.89–0.99	0.02
Cumulative viral burden (copies/mL, $\log_{10}$)	1.55	0.77–3.13	0.22			
History of						
AIDS	1.48	0.51–4.28	0.47			
monotherapy	2.19	0.78–6.15	0.14			
dual therapy	2.16	0.76–6.14	0.15			
cARV	1.09	0.29–4.08	0.90			
NRTI	0.25	0.02–2.92	0.27			
protease inhibitor	1.09	0.29–4.08	0.90			
NNRTI	11.88	3.52–40.14	<0.01			
length of exposure (years)	0.85	0.66–1.09	0.21			
adherence	0.67	0.14–3.19	0.61			
efavirenz	1.54	0.46–5.16	0.48			
nevirapine	7.08	2.29–21.92	0.00	8.95	2.31–34.73	<0.01
efavirenz and nevirapine	2.05	0.46–9.11	0.34			
Comparison of observed and joint effect						
nevirapine exposure	6.38	1.45–28.04	0.01			
nevirapine exposure×nevirapine adherence	1.20	0.23–6.39	0.83			

[a]$P<0.05$.
[b]Due to low numbers of white patients, the composite (white+black) non-Hispanic is used as the reference category.

could not be included in the final model. Thus, a broader class of NNRTIs was examined in the primary analysis and significant associations were confirmed in a separate model including nevirapine. The multivariate analysis showed that previous use of nevirapine (OR 8.95; 95% CI 2.31–34.73; $P<0.01$), being Hispanic (OR 4.76; 95% CI 1.03–21.87; $P=0.04$) and lower CD4% (OR 0.94; 95% CI 0.89–0.99; $P=0.02$) significantly increased the odds of having RAMe (Table 2). Given that nevirapine adherence might modify the effect of nevirapine exposure with regard to the occurrence of RAMe in this cohort, we evaluated the joint effect of these two variables in terms of departure from a multiplicative model by the inclusion of an interaction term in the logistic regression model. The joint effect between these two variables shows a submultiplicative joint effect, where an increase in adherence produces a 1.2-fold decrease in the probability of developing RAMe (Table 2).

Discussion

We report that one-third of perinatally HIV-infected children sequentially treated with antiretroviral regimens available across time had RMs to a new second-generation NNRTI (etravirine), to which they had never been exposed. Previous use of first-generation NNRTIs, mainly nevirapine, and Hispanic ethnicity highly correlated with having RAMe, while higher CD4% was a marker of absence of RAMe.

Seventeen percent and 1.5% of our perinatally HIV-infected population showed an RAMe weighted score between 2.5 and 3.5 and ≥4, respectively. This clearly differs from the rates of RAMe reported in the Thai cohort (47.5% and 49.1%, respectively).[7] These differences could be due to the use of nevirapine as part of cARV or as prevention of mother-to-child transmission, limited accessibility to second-line antiretroviral therapy in developing countries, poor adherence and an increased risk of failing such treatments among infants.[6,10,11] In addition, the lack of access to viral load monitoring and antiretroviral resistance evaluation may lead to a low capacity for detecting viral failure and emergent resistant strains and, therefore, a progressive accumulation of drug RMs.[7,12]

Our results confirm that previous exposure to nevirapine is significantly associated with the probability of acquiring RAMe.[7,12] However, it is important to consider that exposure to nevirapine might not be sufficient to cause etravirine resistance. It is possible that this result arises from an interaction between drug exposure and a diminished antiretroviral adherence.[4,13] Our results indicate a submultiplicative joint effect between these two variables, where an increase in adherence produces a 1.2-fold decrease in the probability of developing RAMe; however, the odds of acquiring RAMe remain significant despite the increase in adherence, indicating there might be other variables that play a role in this causal net.

Our results show that Hispanic ethnicity is significantly associated with the probability of having RAMe. Ethnic health disparities have been well documented in HIV infection and it appears that such disparities are due to the combination of behavioural, cultural and genetic components. Hispanic children and adolescents, compared with whites and blacks, may have more limited access to healthcare, poorer adherence to treatment, reduced clinical follow-up, lack of access to support programmes, limited options to communicate in Spanish and poor retention in clinical care.[14,15] In addition, it is well recognized that HIV along with antiretroviral therapy produces significant metabolic and body composition changes. Some of these changes tend to be more marked among Hispanics, e.g. Gibert *et al.*[16] showed that Hispanic individuals compared with blacks and whites are more susceptible to develop glucose intolerance, insulin resistance and lipoatrophy through the course of antiretroviral therapy. These metabolic alterations, body composition changes and toxicities along with all the social and cultural factors might lead to poor treatment adherence.[17,18]

Finally, we observed that the probability of acquiring RAMe significantly decreased with each unit of increase in CD4%. This is consistent with previous data, where CD4 counts >200 cells/μL were associated with a lower odds of acquiring RAM.[13,17–19] This result suggests that immunological recovery is not just an indicator of therapy success, but at the same time it can be potentially a surrogate marker of therapy adherence and clinical retention.[13,17–19]

This study has a number of limitations: the sample size is small and from one centre; thus, the results may not be generalizable. The presence of detection bias cannot be excluded; sicker patients received more frequent clinical, immunological and genotype evaluation, leading to a possible imprecision in the occurrence of RAMe. Lastly, we cannot rule out the presence of misclassification bias, because the genotype tests have had variable sensitivity and specificity through the years.[20]

In conclusion, this study demonstrates that RAMe in perinatally HIV-infected patients are common, particularly among Hispanic patients, and largely linked to the preceding use of nevirapine. This can potentially impact the efficacy of etravirine in perinatally HIV-infected children and adolescents.

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Transparency declarations

None to declare.

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