Published in final edited form as:

J Acquir Immune Defic Syndr. 2009 June 1; 51(2): 235–237. doi:10.1097/QAI.0b013e31819c155b.

CCR2-64I Polymorphism Is Associated With Lower Maternal HIV-1 Viral Load and Reduced Vertical HIV-1 Transmission

Jennifer M. Mabuka, BS^{*,†}, Romel D. Mackelprang, MS[‡], Barbara Lohman-Payne, PhD^{*,§}, Maxwell Majiwa, BS^{*,†}, Rose Bosire, MBChB, MPH^{*}, Grace John-Stewart, MD, MPH, PhD^{†,‡,§}, Sarah Rowland-Jones, MBChB, MMed^{||,¶}, Julie Overbaugh, PhD[#], and Carey Farquhar, MD, MPH^{‡,§}

^{*}Department of Paediatrics, University of Nairobi, Nairobi, Kenya

[†]Department of Global Health, University of Washington, Seattle, WA

[‡]Department of Epidemiology, University of Washington, Seattle, WA

[§]Department of Medicine, University of Washington, Seattle, WA

^{||}Nuffield Department of Medicine, Oxford University, Oxford, United Kingdom

[¶]Viral Diseases Programme, Medical Research Council, Laboratories, Faraja, Gambia

[#]Division of Human Biology, Fred Hutchinson Cancer, Research Institute, Seattle, WA

To the Editor:

INTRODUCTION

Despite the relatively poor efficiency of HIV-1 infection via CCR2, a single nucleotide polymorphism in the CCR2 coding sequence that results in a valine to isoleucine change has been implicated as a host determinant of HIV-1 transmission and pathogenesis. Several studies have demonstrated an association between the CCR2-64I polymorphism and slower disease progression in adults.¹ Its role in mother-to-child transmission (MTCT) of HIV-1 remains controversial, and its protective effect seems to vary depending on ethnicity and use of antiretroviral drugs for prevention of HIV-1 MTCT.² The CCR2-64I variant is common in the Kenyan population, with a frequency of 25%, enabling us to evaluate associations between the CCR2-64I polymorphism and maternal HIV-1 viral load and to determine the effect of CCR2-64I on HIV-1 MTCT in a cohort in Nairobi.

SUBJECTS AND METHODS

Cohort enrollment information, follow-up, sample collection, and determination of infant HIV status have been reported elsewhere.^{3,4} Briefly, pregnant HIV-1-infected women were enrolled during pregnancy, initiated on zidovudine at 34–36 weeks per Kenyan national guidelines, and followed through delivery and post-partum with their infants for 12 months. Infants were tested at birth, 1 month and every 3 months using HIV-1 DNA and RNA polymerase chain reaction (PCR). Women who intended to breastfeed were genotyped for the CCR2-64I mutation. We used allele-specific amplification refractory mutation system PCR using sequence-specific primers designed to discriminate between single nucleotide

mismatches on their 3' end that coincide with the target mutation. For each reaction, control samples of known genotype were assayed in addition to the study samples.

Group mean comparison *t* tests were used to compare viral loads between CCR2-64I carriers and noncarriers. Cox regression was used to estimate the influence of the alleles on overall transmission and via breastfeeding, with follow-up time censored at last visit or at 12 months. Logistic regression was used to estimate the effect of the polymorphisms on early HIV-1 transmission. We considered 3 genetic models: a *general* model that made no assumptions about the nature of the relationship between the number of CCR2-64I alleles and transmission; a codominant (allele–dose) model that assumed an additive change in risk as the number of CCR2-64I alleles increased; and a dominant model that assumed change in transmission risk as the same whether mothers carried 1 or 2 CCR2-64I alleles. All models were adjusted for maternal viral load at 32 weeks gestation.

RESULTS

Among 272 women enrolled 1999 through 2002, median maternal CD4 T-cell count was 452 cells per microliter [interquartile range (IQR) = 307–452] and median plasma HIV-1 RNA viral load at 32 weeks gestation was 4.8 log₁₀ copies per milliliter (IQR = 4.2–5.3). Median maternal plasma viral load at delivery was 4.1 log₁₀ copies per milliliter (IQR = 3.5–4.7) and was highly correlated with maternal viral load at 32 weeks gestation (Pearson's correlation coefficient = 0.69; *P* < 0.0001). At 1 month after delivery, median breast milk viral load was 2.5 log₁₀ copies per milliliter (IQR = 2.0–3.5). During 12 months of follow-up, 263 of 272 infants (97%) were breastfed, with median breastfeeding duration of 8.5 months (IQR = 4–12).

Two hundred thirty-one women (86%) received short course zidovudine to prevent HIV-1 MTCT and 65 infants (24%) became infected with HIV-1 during follow-up. Fifty-five infants (85%) were infected before 1 month of age and were classified as early infections. Among the 272 mothers, 151 (56%) were homozygous for the wild-type allele, 102 (38%) were heterozygous and 19 (7%) were CCR2-64I homozygous (allele frequency = 25.7%).

At 32-weeks gestation, CCR2-64I carriers had significantly lower mean HIV-1 plasma viral loads than noncarriers (4.48 vs. 4.80 log₁₀ copies/mL, *P* = 0.005). However, at delivery, after short-course zidovudine, this difference in plasma viral load was no longer present (4.14 vs. 3.96 log₁₀ copies/mL, *P* = 0.2). At 1 month after delivery, there was a trend for reduced plasma viral load among CCR2-64I carriers (4.58 vs. 4.81 log₁₀ copies/mL, *P* = 0.07). Based on these differences, we examined whether changes in plasma viral load after zidovudine differed between CCR2-64I carriers and noncarriers. After starting zidovudine, the average decrease in HIV-1 plasma viral load between 32 weeks gestation and delivery was 0.61 log₁₀ copies per milliliter among all women. CCR2-64I carriers experienced a significantly smaller decrease in plasma viral load after zidovudine compared with noncarriers (0.39 vs. 0.77 log₁₀ copies/mL, *P* = 0.001). CCR2-64I carriers also experienced a significantly smaller increase in plasma viral load between delivery and month 1 after zidovudine was stopped (0.42 vs. 0.82 log₁₀ copies/mL, *P* = 0.001). In regression models, the differences in viral load changes between CCR2-64I carriers and noncarriers remained significant even after adjusting for baseline viral load. There were no differences in maternal CD4 T-cell counts, cervical HIV-1 RNA levels, or breast milk HIV-1 viral loads between carriers and noncarriers.

Before 1 year of age, 42 infants (28%) of CCR2-64I noncarriers became infected with HIV-1, 21 infants (21%) of heterozygous carriers acquired HIV-1, and only 2 infants (11%) of homozygous carriers became infected. Under the codominant (allele–dose) model, which assumed transmission risk changed directly with the number of CCR2-64I alleles, we observed

a protective effect for variant alleles. Based on this model, HIV-1 transmission was estimated as 34% less likely among heterozygous carriers and 57% less likely among homozygous carriers when compared with noncarriers [hazard ratio (HR) = 0.66; 95% confidence interval (CI): 0.43 to 0.99, $P = 0.04$ and HR = 0.43; 95% CI: 0.19 to 0.99, $P = 0.04$, respectively] (Table 1). Similarly, we observed a protective trend for carriers of the variant allele under the dominant model, which assumed that the difference in transmission risk was the same for heterozygous and homozygous mothers when compared with wild-type homozygous mothers (HR = 0.61; 95% CI: 0.37 to 1.01, $P = 0.07$). We did not observe protective effects of the 64I allele under the model that made no assumptions about the relationship between the number of 64I alleles and transmission risk (Table 1). After adjusting for maternal HIV-1 viral load at 32 weeks gestation, the protective effect observed for overall transmission retained a similar point estimate but lost statistical significance (HR = 0.75; 95% CI: 0.5 to 1.12, $P = 0.16$). In unadjusted analyses, there was a trend toward protection against transmission before 1 month of age for CCR2-64I heterozygous mothers (odds ratio = 0.61; 95% CI: 0.37 to 1.01, $P = 0.06$). We did not observe any significant associations between the CCR2-64I variant and late HIV-1 transmission via breastfeeding (data not shown).

DISCUSSION

In this Nairobi-based study of vertical HIV-1 transmission, mothers who carried at least 1 CCR2-64I allele had significantly lower viral loads at 32 weeks gestation than noncarriers. Additionally, transmission was less likely among CCR2-64I-carrying mothers, an association that was partially dependent on maternal HIV-1 viral load. Our observations suggest that maternal CCR2-64I may partially protect against MTCT of HIV-1 by reducing baseline plasma HIV-1 viral load.

The mechanism through which CCR2-64I influences HIV-1 viral load remains unclear. CCR2-64I variants and the CCR2 wild type are expressed at similar levels and both function equally well as HIV-1 coreceptors *in vitro* with no apparent effect on expression and coreceptor activity of CCR3, CCR5, and CXCR4.⁵ A possible mechanism for the CCR2-64I effect is through linkage disequilibrium with a point mutation on the CCR5 promoter region (CCR5 59653T), which has been associated with decreased density of the CCR5 receptor on the cell surface, resulting in reduced viral load. Thus, a plausible explanation for the CCR2-64I association viral load observed here is through indirect effects on CCR5 or another key host protein used for HIV-1 replication.

Results regarding CCR2-64I and low viral loads have been inconsistent, possibly due to differences in the average duration of HIV-1 infection between cohorts. The protective effect of CCR2-64I is apparent early in infection when R5 HIV-1 strains, which are associated with delayed onset of AIDS, are dominant.⁶ In our study, CCR2-64I carriers had lower plasma HIV-1 viral loads at 32 weeks of gestation. Although our study was not designed to evaluate duration of infection, women in our cohort were young (median age 24 years), most were asymptomatic with high CD4 counts (median 452 cells/ μ L) and, therefore, may have been in earlier stages of infection.⁴ If these were the case, it could partially explain why CCR2-64I was associated with reduced HIV-1 viral load in our cohort, whereas the association was not observed in other cohorts. We also observed overall protection against HIV-1 transmission and a trend toward protection from early infection, whereas the only other Kenyan study that evaluated associations between the CCR2-64I polymorphism and vertical HIV-1 transmission did not find a protective association.⁷ Use of zidovudine in our cohort potentially unmasked the effect of CCR2 similar to the Kostrikis study by reducing maternal viral loads, which have been reported to modify the effect of single nucleotide polymorphisms.⁸

Antiretroviral prophylaxis has been shown to significantly reduce vertical transmission through reduction of plasma viral load. A recent study showed that the use of antiretrovirals for prevention of MTCT may significantly alter the effect of host genetics on transmission.⁹ In our study, CCR2-64I carriers had lower HIV-1 viral loads at baseline, experienced significantly smaller decreases in plasma HIV-1 viral loads upon prophylactic treatment with zidovudine, and had a significantly smaller viral rebound. One possibility is that generally lower viral replication resulted in low baseline levels and dampened responses to zidovudine.

Strengths of this study include the large number of transmission events overall and frequent HIV-1 testing of infants allowing for determination of timing of infant HIV-1 acquisition. One limitation of this study is that we did not have power to detect associations between the CCR2-64I mutation and late HIV-1 transmission via breastfeeding. Additionally, we have not genotyped CCR2 for the infants in this cohort, so we were unable to assess whether infant CCR2-64I alters susceptibility to HIV-1 infection. Analyzing this association would further enhance our understanding of the CCR2-64I mutation in relation to MTCT. Presence of CCR5 59653T, which is known to be in linkage disequilibrium with CCR2-64I, was also not measured hence it is difficult to rule out its potential effect in the observations made.

In conclusion, the presence of the CCR2-64I allele was associated with reduced viral load and with protection against early HIV-1 transmission among pregnant women who received short course zidovudine. In Kenya and other African countries, where approximately one quarter of individuals carry the variant allele, understanding this genetic mutation may help explain disparities in transmission risk and rates of disease progression and could contribute to vaccine development and other prevention interventions.

Acknowledgments

Supported by US National Institutes of Health grants DE14826, HD23412, HD41879, and TW06080. J.M.M. was a scholar in the AIDS International Training and Research Program, which is supported by the NIH/Fogarty International Center grant D43 TW000007.

REFERENCES

1. Smith MW, Dean M, Carrington M, et al. Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), ALIVE Study. *Science* 1997;277:959–965. [PubMed: 9252328]
2. Ioannidis JP, Contopoulos-Ioannidis DG, Rosenberg PS, et al. Effects of CCR5-delta32 and CCR2-64I alleles on disease progression of perinatally HIV-1-infected children: an international meta-analysis. *AIDS* 2003;17:1631–1638. [PubMed: 12853745]
3. Obimbo EM, Mbori-Ngacha DA, Ochieng JO, et al. Predictors of early mortality in a cohort of human immunodeficiency virus type 1-infected african children. *Pediatr Infect Dis J* 2004;23:536–543. [PubMed: 15194835]
4. Otieno PA, Brown ER, Mbori-Ngacha DA, et al. HIV-1 disease progression in breast-feeding and formula-feeding mothers: a prospective 2-year comparison of T cell subsets, HIV-1 RNA levels, and mortality. *J Infect Dis* 2007;195:220–229. [PubMed: 17191167]
5. Mariani R, Wong S, Mulder LC, et al. CCR2-64I polymorphism is not associated with altered CCR5 expression or coreceptor function. *J Virol* 1999;73:2450–2459. [PubMed: 9971830]
6. Mulherin SA, O'Brien TR, Ioannidis JP, et al. Effects of CCR5-Delta32 and CCR2-64I alleles on HIV-1 disease progression: the protection varies with duration of infection. *AIDS* 2003;17:377–387. [PubMed: 12556692]
7. Brouwer KC, Yang C, Parekh S, et al. Effect of CCR2 chemokine receptor polymorphism on HIV type 1 mother-to-child transmission and child survival in Western Kenya. *AIDS Res Hum Retroviruses* 2005;21:358–362. [PubMed: 15929697]

8. Pedersen BR, Kamwendo D, Blood M, et al. CCR5 haplotypes and mother-to-child HIV transmission in Malawi. PLoS ONE 2007;2(9):e838. [PubMed: 17786209]
9. Singh KK, Hughes MD, Chen J, et al. Associations of chemokine receptor polymorphisms with HIV-1 mother-to-child transmission in sub-Saharan Africa: possible modulation of genetic effects by antiretrovirals. J Acquir Immune Defic Syndr 2008;49:259–265. [PubMed: 18845960]

TABLE 1

Influence of CCR2 Genotype on Mother-Child HIV-1 Transmission

Genetic Model	CCR2 Genotype	HIV-1 Positive/Total (%)	Unadjusted HR or OR (95% CI) [*]	Adjusted HR or OR (95% CI) [*]
Overall transmission				
None [†]	wt/wt	42/150 (28)	Reference	Reference
	wt/64I	21/101 (21)	0.69 (0.41 to 1.15) <i>P</i> = 0.15	0.84 (0.52 to 1.37) <i>P</i> = 0.48
	64I/64I	2/19 (11)	0.36 (0.09 to 1.46) <i>P</i> = 0.15	0.40 (0.09 to 1.84) <i>P</i> = 0.24
Codominant [‡]	wt/wt	42/150 (28)	Reference	Reference
	wt/64I	21/101 (21)	0.66 (0.43 to 0.99) <i>P</i> = 0.04	0.75 (0.50 to 1.12) <i>P</i> = 0.16
	64I/64I	2/19 (11)	—	—
Dominant [§]	wt/wt	42/150 (28)	Reference	Reference
	wt/64I or 64I/64I	23/120 (19)	0.64 (0.39 to 1.04) <i>P</i> = 0.07	0.76 (0.47 to 1.23) <i>P</i> = 0.27
Early transmission				
None [†]	wt/wt	35/150 (23)	Reference	Reference
	wt/64I	19/101 (19)	0.72 (0.38 to 1.28) <i>P</i> = 0.33	0.90 (0.46 to 1.75) <i>P</i> = 0.75
	64I/64I	1/19 (5)	0.19 (0.02 to 1.48) <i>P</i> = 0.11	0.21 (0.02 to 2.00) <i>P</i> = 0.17
Codominant [‡]	wt/wt	35/150 (23)	Reference	Reference
	wt/64I	19/101 (19)	0.61 (0.37 to 1.01) <i>P</i> = 0.06	0.71 (0.42 to 1.19) <i>P</i> = 0.20
	64I/64I	1/19 (5)	—	—
Dominant [§]	wt/wt	35/150 (23)	Reference	Reference
	wt/64I or 64I/64I	20/120 (17)	0.63 (0.34 to 1.18) <i>P</i> = 0.15	0.77 (0.40 to 1.48) <i>P</i> = 0.44

^{*} Hazards ratios and odds ratios were estimated for overall and early transmission, respectively. Adjusted models included plasma HIV-1 viral loads at 32 weeks of gestation.

[†] Model makes no assumptions about relationship between the number of 64I alleles and HIV-1 transmission risk.

[‡] A codominant (allele-dose) model, which assumes that the risk decreased directly with the number of 64I alleles. Risk estimates are comparing wt/64I with wt/wt and 64I/64I with wt/64I.

[§] A dominant model, which assumes that the decrease in risk was the same in pairs in which the mother carried one or the other allele.