CONCISE COMMUNICATION

CCR5 Promoter Polymorphisms in a Kenyan Perinatal Human Immunodeficiency Virus Type 1 Cohort: Association with Increased 2-Year Maternal Mortality

Grace C. John,[1,5] Thomas Bird,[4] Julie Overbaugh,[3,a] Ruth Nduati,[5] Dorothy Mbori-Ngacha,[5] Tim Rostron,[4] Tao Dong,[4] Leondios Kostrikis,[6] Barbra Richardson,[2] and Sarah L. Rowland-Jones[4,a]

Departments of [1]Medicine and [2]Biostatistics, University of Washington, and [3]Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, Washington; [4]Institute of Molecular Medicine, Oxford University, Oxford, United Kingdom; [5]Department of Pediatrics, University of Nairobi, Nairobi, Kenya; [6]Aaron Diamond AIDS Research Center, Rockefeller University, New York, New York

The CCR5 chemokine receptor acts as a coreceptor with CD4 to permit infection by primary macrophage-tropic human immunodeficiency virus type 1 (HIV-1) strains. The CCR5Δ32 mutation, which is associated with resistance to infection in homozygous individuals and delayed disease progression in heterozygous individuals, is rare in Africa, where the HIV-1 epidemic is growing rapidly. Several polymorphisms in the promoter region of CCR5 have been identified, the clinical and functional relevance of which remain poorly defined. We evaluated the effect of 4 CCR5 promoter mutations on systemic and mucosal HIV-1 replication, disease progression, and perinatal transmission in a cohort of 276 HIV-1–seropositive women in Nairobi, Kenya. Mutations at positions 59353, 59402, and 59029 were not associated with effects on mortality, virus load, genital shedding, or transmission in this cohort. However, women with the 59356 C/T genotype had a 3.1-fold increased risk of death during the 2-year follow-up period (95% confidence interval [CI], 1.0–9.5) and a significant increase in vaginal shedding of HIV-1–infected cells (odds ratio, 2.1; 95% CI, 1.0–4.3), compared with women with the 59356 C/C genotype.

The CCR5 coreceptor, as the primary coreceptor of macrophage-tropic human immunodeficiency virus type 1 (HIV-1), is an important determinant of HIV-1 susceptibility and disease progression [1]. A deletion in CCR5 (CCR5Δ32) that abolishes its cell surface expression has been shown to reduce susceptibility to HIV-1 infection in individuals who are homozygous for the deletion [2]. Individuals who are heterozygous for the CCR5Δ32 deletion have delayed disease progression in some but not all studies [3]. This is believed to be related to the reduced surface expression of the CCR5 receptor in cells from CCR5Δ32 heterozygous individuals [4].

More recently, additional polymorphisms in the CCR5 promoter have been identified, some of which appear to have clinical relevance to HIV-1 disease. It is plausible that these promoter mutations may also affect the level of expression of CCR5, which is known to vary even among individuals with wild-type CCR5-coding alleles [4, 5]. To date, however, increased promoter activity has been demonstrated only for the 59029-A allele [6]. Polymorphisms with single nucleotide changes have been described at position 59029, 59353, 59356, and 59402. In one study, a CCR5 promoter variant haplotype with a specific combination of polymorphisms (defined as P1 with 59353-C, 59356-C, and 59402-A) was associated with acceleration of disease progression [7]. The 59029-A allele also has been associated with accelerated disease progression [6]. In contrast, the 59353-C polymorphism was associated with delayed progression to immunosuppression but not to AIDS [8]. The effects of these 2 polymorphisms were analyzed in a recent study that confirmed an association between 59029-A and more rapid disease progression and concluded that 59353-C homozygous individuals were significantly underrepresented among long-term nonprogressors [9]. The latter finding is in contrast to the earlier findings of Easterbrook et al. [8], who noted de-

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Informed consent was obtained from all participants. Guidelines of the US Department of Health and Human Services, the University of Washington Institutional Review Board, and the Kenyatta National Hospital Ethical Review Committee were followed in the conduct of this research.

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[a] J.O. and S.L.R.-J. are Elizabeth Glaser Scientists.

Reprints or correspondence: Dr. Grace John, International AIDS Research and Training Program, Box 359909, University of Washington, Seattle, WA 98195 (gjohn@u.washington.edu).

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layed progression with the 59353-C polymorphism. A detailed analysis of extended CCR5 haplotypes in different population groups demonstrated that the spectrum of haplotypes with disease-modifying effects differed between African Americans and whites [10].

In a recent study combining data from 4 US perinatal HIV-1 cohorts, a polymorphism defined as 59356-T in the CCR5 promoter was shown to be associated with increased perinatal HIV-1 transmission to infants [11]. Among African-American infants whose mothers did not receive zidovudine, there was a highly significant increase in HIV-1 transmission to infants who had the 59356 T/T genotype. This polymorphism was particularly prevalent among African Americans, with an allele frequency of 20.6%, compared with 3.4% among whites and 5.6% among Hispanics.

To our knowledge, the impact of these CCR5 promoter polymorphisms on perinatal HIV-1 transmission and clinical outcome in cohorts in Africa has not been reported previously. We therefore investigated the effect of maternal CCR5 promoter polymorphisms on maternal plasma viremia, shedding of cervical and vaginal HIV-1 infected cells, and 2-year survival in a perinatal HIV-1 transmission cohort in Nairobi, Kenya. The impact of the 59356-T mutation on infant susceptibility to HIV-1 transmission also was assessed.

Methods

Study cohort. The recruitment and follow-up of women and infants in the cohort have been described elsewhere [12]. HIV-1–seropositive women were enrolled into the study during pregnancy and underwent genital sampling for HIV-1 provirus at 32 weeks of gestation [13]. At that time, blood was obtained for determination of T cell subsets and plasma HIV-1 RNA levels. Mother-infant pairs were followed monthly during the first year after birth and every 3 months during the second year. Infant HIV-1 infection status was determined by use of HIV-1 DNA polymerase chain reaction (PCR) assays of specimens obtained at 6 and 14 weeks and every 3 months thereafter for the first 2 years of life. Assays for maternal plasma HIV-1 RNA and cervical and vaginal HIV-1 proviral DNA have been described elsewhere [12, 13].

Polymorphism assays. Maternal DNA was extracted from lymphocytes processed from blood obtained at week 32 of gestation. Maternal specimens were assessed for previously defined CCR5 polymorphisms at position 59353 (T/C), 59356 (T/C), 59402 (A/G), and 59029 (A/G), together with the CCR5Δ32 deletion. Because homozygosity for the 59356-T mutation recently had been reported to be associated with increased perinatal transmission in infants born to infected mothers in the Women and Infants Transmission Study cohort [11], we assessed infant specimens for this polymorphism and determined its associated risk of transmission. The assays were conducted using an amplification-refractory mutation system PCR (ARMS-PCR) technique with sequence-specific primers, as described elsewhere [9].

To confirm the ARMS-PCR results, a selection of 16 maternal DNA samples with different PCR genotyping results were subjected to sequencing of the CCR5 promoter region encompassing the 59353, 59356, and 59402 polymorphisms. The results of ARMS-PCR were confirmed by sequence analysis in all cases.

Statistical analysis. Analyses were conducted comparing individuals with the polymorphism (i.e., including both those who were heterozygous and those who were homozygous for the less prevalent ["mutant"] genotype) versus the most prevalent homozygous genotype. For polymorphisms in which the homozygous mutant frequency was <5% of that of the cohort, comparisons were restricted to comparisons of heterozygous versus most prevalent homozygous genotype. The effect of maternal CCR5 promoter polymorphisms on plasma virus loads greater than the median, cervical and vaginal HIV-1 DNA detection, infant HIV-1 infection, and 2-year maternal mortality status was determined using χ^2 tests. The effect on maternal survival was assessed using Kaplan-Meier survival analysis, and risk of maternal mortality was determined using Cox regression analysis. The effect of the infant 59356-T polymorphism on infant infection was assessed using χ^2 tests. The effect of the polymorphism on infant survival was assessed with Kaplan-Meier survival and Cox regression analysis.

Results

Coreceptor allele and genotype frequencies in the cohort. Of 276 women tested for the CCR5Δ32 mutation, 275 had the wild-type polymorphism and 1 (0.04%) was heterozygous for the mutation. The CCR5 promoter was examined for mutations at positions 59353 (C/T), 59402 (G/A), 52029 (G/A), and 59356 (C/T). The allele frequency was 0.39 for 59353-T, 0.11 for 59402-A, and 0.48 for 59029-A. Among 243 women, the allele frequency for 59356-T was 0.13, and the genotype frequencies at this position were 1% TT, 24% TC, and 75% CC. Among 251 infants in the cohort for whom genotyping results were available, the allele frequency of the CCR5 promoter 59356-T polymorphism was 0.12, with genotype frequencies of 2% TT, 18% CT, and 79% CC. Infants heterozygous for the 59356-T polymorphism were significantly more likely to have a mother with the 59356 C/T genotype rather than the 59356 C/C genotype (odds ratio [OR], 3.7; 95% confidence interval [CI], 1.4–9.5).

Coreceptor mutations and plasma, cervical, and vaginal HIV-1. Sixteen (29%) of 56 women with the 59356 C/T genotype had detectable vaginal HIV-1 DNA versus 26 (16%) of 163 women with the 59356 C/C genotype ($P = .04$). Women who were heterozygous for 59356-T in the CCR5 promoter had increased levels of vaginal (OR, 2.1; 95% CI, 1.0–4.3) but not cervical HIV-1–infected cells (table 1). Plasma viral RNA levels did not differ significantly in women with different polymorphisms in the CCR5 promoter.

Perinatal HIV-1 transmission. None of the maternal CCR5 promoter polymorphisms was significantly associated with increased perinatal HIV-1 transmission (table 1). Only 6 infants were homozygous for the 59356-T polymorphism, of whom 2 were HIV-1 infected (33%). The risk of HIV-1 infection was higher among infants with the 59356 T/T genotype than among heterozygous infants (9 [20%] of 46 59356 C/T infants were

Table 1. Effect of maternal coreceptor mutations on maternal human immunodeficiency virus (HIV) plasma load, genital shedding of HIV-infected cells, and perinatal transmission of HIV in a perinatal HIV-1 transmission cohort in Nairobi, Kenya.

CCR5 promoter polymorphisms	HIV-1 RNA level >43,000 copies/mL[a]	Cervical HIV-1 DNA	Vaginal HIV-1 DNA	Infant HIV-1 infection	Perinatal HIV-1 transmission
59029-A	1.1 (0.6–2.0)	1.4 (0.7–2.8)	0.8 (0.4–1.7)	1.2 (0.6–2.5)	
59353-T	1.1 (0.6–2.0)	1.2 (0.7–2.2)	1.2 (0.6–2.4)	1.1 (0.6–2.0)	1.0 (0.4-2.9)
59356-T	0.8 (0.4–1.5)	1.1 (0.6–2.0)	2.1 (1.0–4.3)	1.2 (0.6–2.5)	1.3 (0.7-2.7)
59402-G	0.8 (0.4–1.5)	1.2 (0.6–2.3)	0.9 (0.4–2.0)	1.3 (0.6–2.6)	1.2 (0.6-2.4)

NOTE. Data are odds ratio (95% confidence interval).
[a] Median virus load for the cohort.

HIV-1 infected) or 59356 C/C infants (49 [25%] of 197 59356 C/C infants were infected); however, there was an insufficient number of infants with the 59356 T/T genotype to determine an association with perinatal HIV-1 transmission in this cohort.

Maternal postpartum survival. The perinatal HIV-1 cohort was designed to follow mother-infant pairs for 2 years after birth. This provided the opportunity to study maternal mortality in relationship to coreceptor polymorphisms. The relative hazard for maternal mortality associated with the 59356-T mutation was 3.1 (95% CI, 1.0–9.5; $P = .05$). Women who were heterozygous for the 59356-T polymorphism had significantly decreased survival rates, as determined by Kaplan-Meier analysis ($P = .04$; figure 1). Mutations at position 59402, 59029, and 59353 of the CCR5 promoter were not associated with changes in maternal survival (data not shown). Maternal mortality over the 2 years of postpartum follow-up (from index pregnancy) was significantly associated with immunosuppression and plasma HIV-1 RNA burden during pregnancy and with randomization to the breast-feeding (vs. formula-feeding) arm of the study [14]. When we controlled for plasma virus load and randomization to breast-feeding, the relationship between the heterozygous 59356-T mutation and mortality remained significant (relative risk, 3.1; 95% CI, 1.0–9.6, $P = .05$).

Because most infants in the cohort were not infected with HIV-1, there was not power to determine the effect of the 59356-T mutation in infants on disease progression in HIV-1–infected infants. Among 58 HIV-1–infected infants for whom we had data on CCR5 promoter polymorphisms, only 9 were heterozygous for the 59356-T mutation.

Discussion

We observed significantly decreased 2-year postpartum survival in mothers with the 59356 C/T genotype. In our cohort of HIV-1–seropositive women, other CCR5 promoter polymorphisms were not associated with significant effects on 2-year survival. In addition, women with the 59356 C/T genotype had significantly increased vaginal shedding of HIV-1, which might be expected to increase their potential infectivity. Although changes at positions 59029 and 59353 in the CCR5 promoter have been associated with accelerated disease progression [6, 8, 9], the effect of the 59356 polymorphism on survival has not been previously defined. One way to explain our observation of decreased survival and increased shedding among women with this mutation is that the mutation may increase expression of CCR5 and thus influence disease progression. Increased expression of CCR5 could also explain the observed increase in shedding of vaginal HIV-1–infected cells in women with this mutation. However, it is somewhat surprising that we did not see increased plasma virus load or shedding of cervical HIV-1–infected cells in women with this polymorphism. This may suggest that there are cell- or tissue-specific effects of this mutation on expression, as has been suggested by Patterson et al. [15].

Although our study did not find a relationship between the 59356 C/T genotype and perinatal HIV-1 transmission, our findings are consistent with those of Kostrikis et al. [11], who demonstrated that African-American infants homozygous for the 59356-T mutation in 4 US perinatal HIV-1 transmission cohorts had enhanced susceptibility to HIV-1 infection. That finding suggests that the mutation has effects on cellular entry,

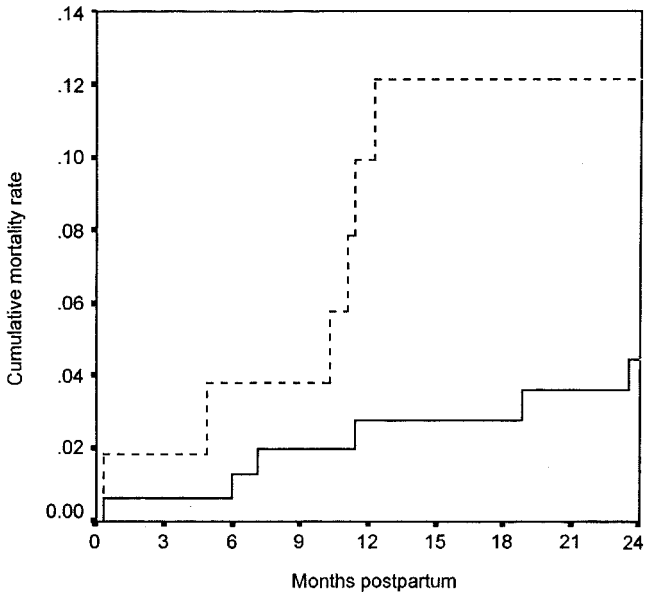

Figure 1. Mortality among human immunodeficiency virus type 1–infected mothers in Nairobi, Kenya, by 59356-T polymorphism. *Dashed line,* wild type; *solid line,* heterozygous.

replication, or virus release. In the study by Kostrikis et al., there were 667 infants, 38 of whom were homozygous for the 59356-T mutation. In our cohort, the allele frequency for the 59356-T mutation was 0.13, compared with 0.21 in the Kostrikis cohort, and only 6 infants were homozygous for the mutation, making it impossible to assess effect of the homozygous mutation on perinatal transmission. In addition, our observation of increased vaginal shedding of HIV-1 among women with the 59356 C/T genotype may partially explain the observation of increased perinatal HIV-1 transmission seen among infants with the 59356 T/T genotype in the Kostrikis study. Women with the 59356 C/T mutation would be expected to have increased vaginal shedding, with the potential for increased perinatal HIV-1 transmission, and these mothers also would have increased likelihood of having infants with the 59356 T/T genotype.

Despite a lower allele frequency, which limited our ability to assess the 59356-T homozygous mutation, we observed 2-year mortality and vaginal shedding effects among women who were heterozygous for the 59356-T mutation that were not seen for any of the other CCR5 promoter polymorphisms (59029, 59353, and 59402) we assessed. In cohorts with higher allele frequencies for the 59356 polymorphism, it will be important to assess the effect on disease progression and HIV-1 replication among individuals who are homozygous for the mutation. Our study adds to the growing evidence that CCR5 promoter polymorphisms influence HIV-1 pathogenesis, infectivity, and susceptibility, but it is difficult to draw conclusions regarding their mechanism of action from epidemiologic studies. In vitro studies of the CCR5 promoter 59356-T polymorphism, using relevant cell types found in lymph nodes versus genital compartments, will be necessary to determine its effect on HIV-1 entry and replication.

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