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Impact of Natural Chemokine Receptor Polymorphisms on Perinatal Transmission of Human Immunodeficiency Virus Type 1

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The rate of progression of human immunodeficiency virus type 1 (HIV-1) disease exhibits an extraordinary variation among individuals. The most commonly transmitted strains of HIV-1 fuse with their target cells after interacting with CD4 and the CC-chemokine receptor 5 (*CCR5*). CCR5 is the major co-receptor for the most commonly transmitted strains of HIV-1 (Alkhatib, '96; Choe, '96; Deng, '96; Doranz, '96; Dragic, '96). Over the past few years, several mutations in *CCR5* have been identified as natural genetic polymorphisms that are able to influence the probability of acquiring HIV-1 infection or to affect the rate of disease progression, in infected adults or infants (Buseyne, '88; Dean, '96; Huang, '96; Liu, '96; Samson, '96; Michael, '97; Zimmerman, '97; Shearer, '98). A deletion of 32-nucleotides in the *CCR5* gene (denoted *CCR5-Δ32*) results in a truncated protein that is not expressed on the cell surface; individuals homozygous for this deletion have an absolute resistance to infection by CCR5-using HIV-1 variants (Dean, '96; Liu, '96; Samson, '96), whereas heterozygotes for *CCR5-Δ32* do not resist HIV-1 infection, but progress more frequently to acquired immunodeficiency syndrome (AIDS). Another mutation in a closely linked chemokine receptor gene, *CCR2*, also affects HIV-1 disease progression. This mutation, denoted CCR2-*64I*, is a G-to-A substitution that results in a replacement of valine with isoleucine at position 64 of the CCR2 protein (Smith, '97). The homozygous CCR2-*64I/64I* phenotype has no effect on HIV-1 transmission but is associated with a delayed progression to disease (Smith, '97; Kostrikis, '98). Several genetic variations have recently been identified within the CCR5 regulatory region (Mummidi, '97, '98; Kostrikis, '98; Martin, '98; McDermott, '98), some of which have been reported recently to affect the rate of disease progression in adults (Martin, '98; McDermott, '98; Mummidi, '98).

We studied the impact of five *CCR5* and *CCR2* polymorphisms *-CCR5-59353-T/C*, *CCR5-59356-C/T CCR5-59402-A/G*, CCR5-Δ32, and *CCR2-64I-* in the setting of maternal-infant HIV-1 transmission from the following four pediatric cohorts in United States: Women and Infants Transmission Study Cohort (WITS), ARIEL Project Cohort, New York City-Western New England Cohort and Newark Perinatal Cohort (Kostrikis, '99). Figure 1A demonstrates the genomic organization of the *CCR2* and *CCR5* genes on chromosome 3, as well as the location of the above polymorphic sites. The overall racial distribution for the combined cohorts was 47.9% African-Americans, 13.5% Caucasians, 34.1% Hispanics, and 4.5% other racial groups (Fig. 1B). The clinical significance of each genotype was assessed by measuring whether it influenced the rate of perinatal HIV-1 transmission among 667 AZT-untreated mother-infant pairs (554 uninfected and 113 HIV-1 infected). We have evaluated each mutation by comparing the genotype and mutant allele frequencies between HIV-1-infected and -uninfected groups and by comparing the fraction of HIV-1-infected children among the different genotypes. We found the *CCR5-Δ32* deletion to be significantly more prevalent among Caucasians than among African-Americans and Hispanic individuals, consistent with previous report. Untreated *CCR5-Δ32* heterozygotes had lower HIV-1 transmission rates than were found in *CCR5* wild-type homozygous infants, but this difference was not statistically significant. The distribution of the *CCR2-64I* genotypes was similar among the three racial groups, consistent with previous reports. In the untreated group, there was no effect of *CCR2-64I* on HIV-1 transmission. The frequency of the *CCR5-59353C* allele was high in the combined population, but was significantly lower among African-Americans than in Hispanic individuals and Caucasians. In the AZT-untreated group, the *CCR5-59353C* allele had no observable effect on transmission. The frequency of the *CCR5-59402G* mutant allele was significantly lower among African-Americans than in the Caucasian and Hispanic groups. In the untreated group, there was a trend for a reduced HIV-1 transmission rate to infants who are *CCR5-59402G* mutant homozygotes, compared with *CCR5-59402A* wild-type homozygous infants.

Most significantly, we found that the mutant *CCR5-59356T* allele was relatively common in African-Americans (20.6% allele frequency among 552 infants), and rare in Caucasians and Hispanics (3.4% and 5.6% of 174 and 458 infants, respectively; $P < 0.001$). In fact,

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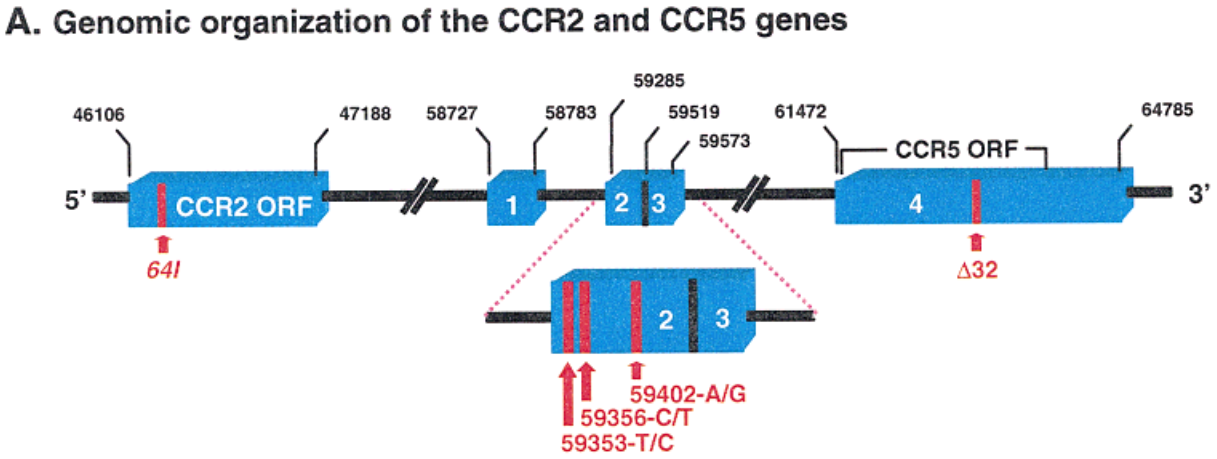
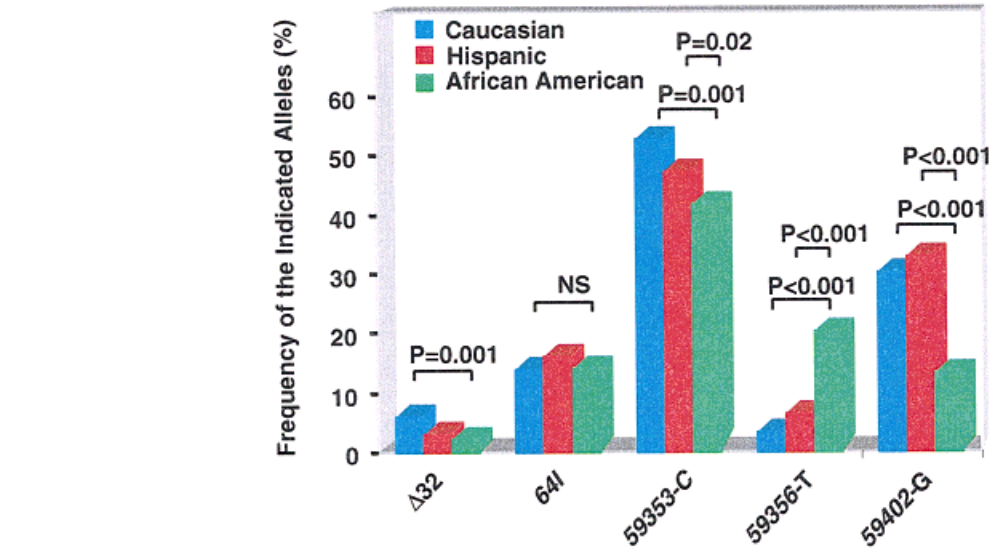

A. Genomic organization of the CCR2 and CCR5 genes
46106
47188
58727
58783
59285
59519
59573
61472
64785
5'
CCR2 ORF
CCR5 ORF
3'
1
2
3
4
64I
Δ32
59402-A/G
59356-C/T
59353-T/C


B. Racial distributions of the CCR2 and CCR5 polymorphisms
Frequency of the Indicated Alleles (%)
Caucasian
Hispanic
African American
P=0.02
P=0.001
P<0.001
P<0.001
P<0.001
NS
P=0.001
Δ32
64I
59353-C
59356-T
59402-G

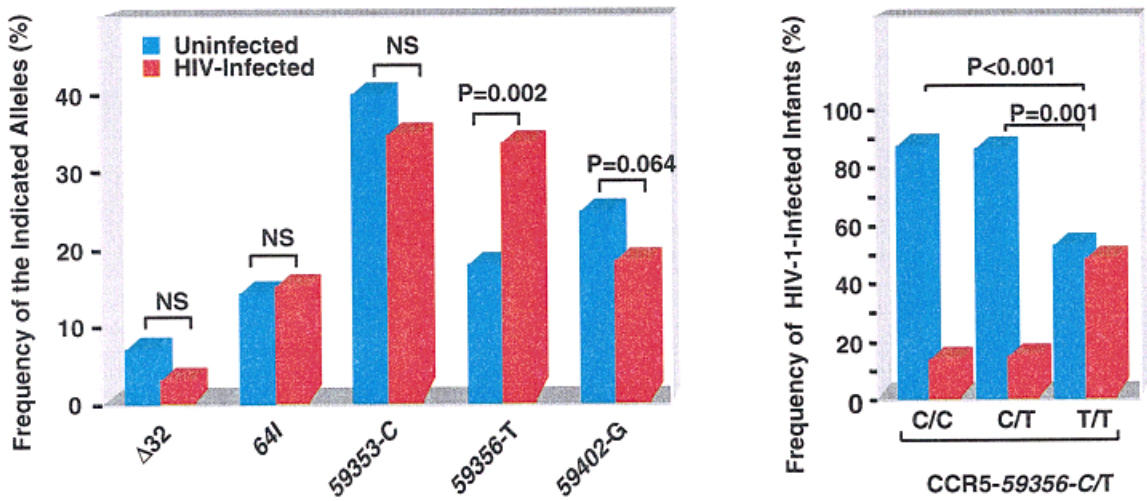

C. Significance of CCR5 and CCR2 polymorphisms on perinatal HIV-1 transmission
Frequency of the Indicated Alleles (%)
Uninfected
HIV-Infected
NS
P=0.002
P=0.064
Δ32
64I
59353-C
59356-T
59402-G
Frequency of HIV-1-Infected Infants (%)
P<0.001
P=0.001
C/C
C/T
T/T
CCR5-59356-C/T

Figure 1.

CCR5-59356T mutant homozygotes were most found among African-Americans: 35 of the 38 infants mutant homozygous for *CCR5-59356T*, 35 were African-Americans. By comparing the fractions of untreated, HIV-1-infected infants in the three *CCR5-59356* genotypes, we found a significantly higher rate of transmission among *CCR5-59356-T* mutant homozygotes (47.6% of 21 *CCR5-59356-T* mutant homozygote infants), compared with both *CCR5-59356-A* wild-type homozygotes (13.4% of 187; $P < 0.001$) and *CCR5-59356* heterozygotes (14.1% of 71; $P = 0.001$). In other words, about one-half of the untreated infants in the four combined cohorts who are homozygotes for the *CCR5-59356-T* mutation are infected with HIV-1, an unexpectedly large fraction. Infants who are *CCR5-59356-T* mutant homozygotes are associated with an increased relative risk of HIV-1 infection of 5.9 (95% confidence interval, 2.3 to 15.3) ($P < 0.001$). The enhancing effect of the *CCR5-59356-T* mutation on HIV-1 transmission was not observed in the AZT-treated group.

The central finding from our study was that the *CCR5-59356T/T* mutant genotype, which was predominantly found in African-Americans, was associated with a significantly higher rate of perinatal HIV-1 infection. Although the frequency of the *CCR5-59356T/T* mutant genotype among African-Americans was not sufficient to cause a significant overall increase in the rate of perinatal HIV-1 infection among African-Americans, it remains possible that higher frequencies of the *CCR5-59356T/T* mutant genotype in African populations may contribute to the relatively high rate of perinatal HIV infection in Africa. We are currently expanding our studies in two pediatric cohorts from Africa: the pediatric Cohort in Butare, Rwanda, and the South African Pediatric Cohort in Soweto, South Africa. We are also investigating the biological basis for this adverse epidemiological effect of the *CCR5-59356T* allelic variant. Using gel-shift assays and protein extracts $CD4^+$ and $CD8^+$ T lymphocytes, monocytes, and dendritic cells, we showed that the *CCR5-59356T* mutation creates a binding site for an unknown cellular protein. The *CCR5-59356T*-binding protein was present in T cells and monocytes from all the donors tested, including African-American individuals who are *CCR5-59356T/T* mutant homozygotes. This preliminary finding may suggest that any donor-dependent influence of the *CCR5-59356T*-binding protein on HIV-1 transmission is more likely to be mediated by the presence or absence of its binding site within the *CCR5* regulatory region, rather than whether or not the factor itself is expressed.

Fig. 1. A: Genomic organization of the *CCR2* and *CCR5* genes on chromosome 3. The location of polymorphic sites in the regulatory region of *CCR5* (*59029-G/A*, *59353-T/C*, *59356-C/T*, and *59402-A/G*) and in the coding regions of *CCR5* (Δ32) and *CCR2* (64I) genes are indicated by red arrows. Open boxes indicate the noncoding exons and the open reading frames (ORF); lines signify the introns. Exons and mutations are numbered based on the nucleotide position of the unpublished sequence with GenBank accession number U95626. **B:** Frequency of the *CCR2* and *CCR5* polymorphisms among Caucasians, Hispanics, and African-Americans from all the pediatric cohorts. **C:** Frequencies of the mutant *CCR5-Δ32*, *CCR2-64I*, *CCR5-59353-C*, *CCR5-59356-T*, and *CCR5-59402-G* alleles in HIV-1-infected and -uninfected infants. **Left:** The frequency of the *CCR5-59356-T* genetic variant is significantly higher in HIV-1-infected than in -uninfected infants ($P = 0.002$), whereas there was a trend in the association of the CCR5-59402-G allele with a decreased rate of perinatal transmission ($P = 0.064$). There is no significant difference in the frequencies of the *CCR5-Δ32*, *CCR2-64I*, and *CCR5-59353-C* alleles between the two groups. **Right:** Untreated African-American *CCR5-59356-T* mutant homozygotes have a highly significantly ($P < 0.001$) increased rate of HIV-1 transmission compared with *CCR5-59356-C* wild-type homozygotes or heterozygotes.

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