Concordance between the CC Chemokine Receptor 5 Genetic Determinants That Alter Risks of Transmission and Disease Progression in Children Exposed Perinatally to Human Immunodeficiency Virus

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If CC chemokine receptor 5 (CCR5)-dependent mechanisms at the time of initial virus exposure are important determinants of virus entry and disease outcome, then the polymorphisms in CCR5 that influence risk of transmission and disease progression should be similar; this hypothesis was tested in a cohort of 649 Argentinean children exposed perinatally to human immunodeficiency virus type 1 (HIV-1). Two lines of evidence support this hypothesis. First, CCR5 haplotype pairs associated with enhanced risk of transmission were the chief predators of a faster disease course. Second, some of the haplotype pairs associated with altered rates of transmission and disease progression in children were similar to those that we previously found influenced outcome in European American adults. This concordance suggests that CCR5 haplotypes may serve as genetic rheostats that influence events occurring shortly after initial virus exposure, dictating not only virus entry but, by extension, also the extent of early viral replication.

Several key conceptual issues regarding the role of host factors in the pathogenesis of human immunodeficiency virus type 1 (HIV-1) infection remain unresolved, in particular the relationship between expression levels of CC chemokine receptor 5 (CCR5), the major coreceptor for the virus’s entry into cells [1], and HIV-1 pathogenesis. The importance of understanding this relationship stems from the finding that individuals who are homozygous for an inactivating mutation (i.e., a 32-bp deletion [A32]) in CCR5 lack expression of the receptor and thereby resist infection [2–5]. Because of this seminal finding, and the observation that there is significant interindividual variation in CCR5 expression levels [6, 7], intense research efforts have been expended to determine whether this variation is also associated with differences in transmission and clinical outcome.

The findings of several studies of nonhuman primate models of HIV-1 demonstrate that disease outcome in lentivirus infection is largely dictated by events that determine the extent of viral replication that occurs immediately after infection [8–10]. Host, rather than viral, factors are thought to control these very early events; however, the precise nature of these events or the exact identities of these host factors remain elusive. Thus, from a pathophysiologic perspective, the time at which CCR5 expression levels would be most relevant is during and shortly after HIV-1 target cells come into contact with virus. Such target cells during sexual transmission include CD4+ T cells in the draining lymph nodes, where immature mucosal dendritic cells carrying HIV-1 arrive and initiate both a primary antiviral immune response and a vigorous productive infection of T cells, allowing for systemic distribution of HIV-1 [1, 11–13]. However, determining the expression level of CCR5 in HIV-1 target cells or tissues during this pathophysiologically highly relevant but limited time window (i.e., the very early stages of...
infection) is extremely difficult. Furthermore, expression levels of CCR5 in easily accessible cells, such as peripheral blood mononuclear cells, may be poor surrogates for expression levels in target cells that play important roles during the early phases of HIV-1 infection [1, 14, 15]. Also, after infection has occurred, CCR5 levels may be influenced significantly by several other factors, including immune activation [16].

Given these significant limitations, an alternative and highly informative approach to understanding the relationship between CCR5 expression and HIV pathogenesis is to define the association between polymorphisms that may affect CCR5 gene expression and the risk of transmission and/or clinical progression rate, which is an approach widely adopted [3–5, 17–38]. We hypothesized that if the CCR5 haplotypes that influence transmission of HIV and progression to disease operate through interrelated mechanisms, then the following condition should be affirmed: the CCR5 polymorphisms that alter the risk of transmission should correspond to those that eventually affect clinical progression rate.

Genetic-epidemiologic studies to determine host genetic factors that influence HIV transmission ideally should be in genetically well-defined and matched control subjects with comparable levels of risk exposure. Likewise, the genetic determinants of HIV disease progression ideally should be examined in individuals who have similar modes of transmission in addition to well-defined estimates of time of transmission. However, identifying such cohorts in adult populations is challenging.

We tested our hypothesis in a cohort of children exposed perinatally to HIV-1. Perinatally acquired HIV-1 infection is an unfortunate, highly understudied, yet exceptionally valuable model to identify host determinants of HIV-1 transmission and progression to disease for 4 reasons. First, HIV-1 is naturally transmitted to 13%–48% of children born to infected mothers [39]. Thus, compared with the risk of HIV transmission after a single sexual exposure (~0.01%–1%) [40], the risk of mother-to-child transmission is very high. Second, the uninfected children of HIV-infected mothers who did not receive preventive therapy with zidovudine [41] are an ideal control population of high-risk exposed yet uninfected individuals, against which to well-defined estimates of time of transmission. However, identifying such cohorts in adult populations is challenging.

We studied a cohort of children exposed perinatally to HIV-1 from Buenos Aires. An important consideration for any genetic-epidemiologic study is that the population studied be genetically similar. In this regard, on the basis of demographic history, as well as genetic studies [53], the population of Argentina is widely regarded to have one of the most European-like populations of all Latin American countries. The vast majority of Argentineans are descendants of individuals from southern Europe, primarily from Spain and Italy, there is little admixture with Amerindians, and there is no substantial population of individuals of African origin. In the context of this background, as well as the fact that the vast majority of the children were from hospital sources in Buenos Aires, suggests that the HIV-positive and HIV-negative children who we studied were demographically and ethnically very similar.

DNA was available from 649 children perinatally exposed to HIV-1 from 1986 to 1998. The HIV-1–infected children were followed at a tertiary care, academic, pediatric hospital (Hospital de Pediatría "J. P. Garrahan") in Buenos Aires. Physicians from different medical centers, primarily in Buenos Aires, referred children ≤18 months old to this hospital for early diagnosis or those >18 months old because the children had an illness compatible with a diagnosis of HIV infection and/or needed specialized medical care. Therefore, we recruited the following subjects: all children (either HIV-positive or HIV-negative) born to HIV-positive mothers in 2 maternity hospitals that are closely affiliated with this tertiary-care center, and additional HIV-positive children (born to HIV-positive mothers) who were referred to this tertiary-care center. A total of 347 HIV-positive and 302 HIV-negative children born to HIV-1–positive mothers were followed-up prospectively. This higher proportion of infected than uninfected children is not indicative of transmission rate, because ascertainment was skewed toward infected children. Thus, the makeup of this cohort is similar to cohorts of highly HIV-exposed adults, some of whom remain uninfected, whereas others become infected [5].

HIV-1 infection status, AIDS definitions, and stage of immune suppression were established according to the 1994 criteria of the Centers for Disease Control and Prevention classification for children [54]. The zidovudine prophylaxis provided (or available) to mother-infant pairs was done according to the AIDS Clinical Trials Group Protocol 076 [41] and was considered to be complete in 110
A classification and genotypic features of CCR5 haplotypes. The genotypic characteristics of haplotypes within each haplogroup at the polymorphic positions CCR2-V64I (G190A); CCR5 A→2733G, G→2554T, G→2459A, T→2135C, C→2132T, A→2086G, and C→1835T; and wild-type (Wt) open-reading frame (ORF)/Δ32 are shown. HHA is the ancestral haplotype [25, 59], and changes relative to the nucleotide sequence in HHA are in boldface. Number (#) system refers to the 3 commonly used CCR5 numbering systems: 1 is based on GenBank accession nos. AF031236 and AF031237 [56], whereas 2 is based on GenBank accession no. U95626, and 3 is the new numbering system wherein the first nucleotide of the CCR5 translational start site is designated as +1 and the nucleotide immediately upstream as −1 [29, 59].

B. Schematic illustration of the CCR5 HHE/HHG*2 haplotype pair. Homozygosity refers to 2 haplotypes of the same haplogroup, and heterozygosity indicates 2 haplotypes from 2 different haplogroups.

Figure 1. Schematic illustration of CC chemokine receptor 5 (CCR5) haplotypes. (A) Classification and genotypic features of CCR5 haplotypes. (B) Schematic illustration of the CCR5 HHE/HHG*2 haplotype pair. Homozygosity refers to 2 haplotypes of the same haplogroup, and heterozygosity indicates 2 haplotypes from 2 different haplogroups.

(92 uninfected and 18 infected children), partial (mother or child) in 17 (2 uninfected and 15 infected), and absent in 466 (160 uninfected and 306 infected) children. For statistical analysis, mother-infant pairs that received complete or partial zidovudine prophylaxis were pooled. Information regarding zidovudine prophylaxis was unavailable for 56 mother-child pairs (48 uninfected and 8 infected), and they were not included in the statistical analyses that accounted for the effects of zidovudine. After 1992, all infected children received antiretroviral therapy, according to the recommended guidelines [55]. The median follow-up was 4.08 years; 55.6% of this cohort progressed to AIDS, and 7.2% died during the study period, which ended 1 January 1999. The clinical care of the patients was under the supervision of a single medical-care provider (R.B).

**Genotyping analysis.** CCR5 is a highly polymorphic gene [1, 2, 21, 26–30, 56–59]. CCR5 sequences that differ from each other are referred to as alleles (i.e., CCR5 alleles). A CCR5 allele and polymorphisms in the neighboring sequences, including those in CCR2, a coreceptor situated ~8 kb upstream of CCR5, which are tightly linked together, define a CCR5 haplotype [25, 26, 56, 59]. The cohorts were genotyped for the CCR5 and CCR2 polymorphisms indicated in figure 1A. Genotyping methods and haplotype classification were as described elsewhere [25, 59]; additional details can be obtained from S.K.A.). In this classification system, CCR5 haplotypes are grouped into 1 of 7 human haplogroups (HH): HHA, HHB, HHC, HHD, HHE, HHF (F*1 and F*2), and HHG (G*1 and G*2) [25, 59]. A schematic illustration of the arrangement of CCR5 polymorphisms on chromosome 3 is shown in figure 1B. Differences between the genotypic features of the haplotypes and the classification system used by other investigators [21, 27, 28, 30, 37] and those used in this study have been discussed previously [25, 59] and are further addressed below.

**Statistical analysis.** The overall association between possession of a CCR5 haplotype or haplotype pair and risk of transmission was evaluated by Fisher’s exact test (SAS, version 8.0; SAS Institute). When an overall difference was observed, we adopted the strategy similar to the least significance difference method proposed by Fisher [60], to determine which haplotype or haplotype pair was contributing to the overall effect. This strategy of first defining an overall test of association before testing individual haplotypes minimizes the risk of false-positive results (type I errors). In this strategy, a nominal variable that classified subjects into 1 of 33 classes, depending on their haplotype pair composition (table 1), or 1 of 9 classes, depending on their haplotype composition (table 2), was created. This nominal variable was used in multivariable logistic models to examine the association between CCR5 haplotypes or haplotype pairs and risk of transmission. To maintain power, the classes of haplotype pairs that had <9 individuals were pooled and were included in the model. A χ² test for homogeneity was used to determine whether the use of zidovudine or other antiretroviral therapy was proportionally similar among children who had different haplotypes or haplotype pairs. The risk of transmission in the entire cohort was adjusted for the transmission-inhibiting effects of zidovudine. For haplotypes and haplotype pairs that were associated with differences in the risk of transmission, time curves for progression to AIDS (1994 criterion) were prepared by the Kaplan-Meier method, using SAS (version 6.12; SAS Institute). Relative hazards were calculated with Cox proportional hazard models, as described elsewhere [25, 26, 38].

**Results**

**Transmission-modifying CCR5 genetic determinants.** We used an evolutionary-based classification of CCR5 polymorphisms [25, 59] to define the 2 CCR5 haplotypes found in each child (table 1) and used this information to determine their combined HIV transmission- and/or disease-modifying phenotypic effect. Similar to results found in European American adults, but in contrast to those found in African Americans...
CC chemokine receptor 5 haplotype pairs in Argentinean children exposed perinatally to human immunodeficiency virus (HIV).

<table>
<thead>
<tr>
<th>Haplotype pair</th>
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<th>HIV+ (n = 347)</th>
<th>All HIV (n = 160)</th>
<th>HIV+ (n = 306)</th>
<th>Prophylaxis HIV (n = 94)</th>
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NOTE: Data are no. of children. Prophylaxis refers to prophylaxis with zidovudine. HIV+, HIV-positive children; HIV−, HIV-negative children.
a Includes 48 mother-child pairs without prophylaxis information.
b Includes 8 mother-child pairs without prophylaxis information.

[25], CCR5 haplotype pairs HHC/HHE, HHC/HHC, and HHE/HHE were the 3 most common pairs found in Argentinean children and accounted for nearly 40% of the 33 haplotype pairs identified (table 1).

There was a strong association between genetic variation at the CCR5 locus and susceptibility to perinatal transmission of HIV-1, regardless of zidovudine use. By Fisher’s exact test (2×9 table analysis), the difference between the frequencies of the 9 CCR5 haplotypes in HIV-positive and HIV-negative children in either the entire cohort or only those who did not receive prophylaxis was highly significant (P < .0001; table 2). Similarly, there was a highly significant difference between the frequencies of the CCR5 haplotype pairs (table 1) in the HIV-positive and HIV-negative children (P < .0001, Fisher’s exact test for the entire group or those who did not receive prophylaxis). Thus, we rejected the hypothesis of no association between the risk of transmission and the possession of particular CCR5 haplotype pairs.

Because of this overall strong association, we next determined which of the CCR5 haplotypes differed significantly in frequency between the uninfected and infected groups (table 2). The prevalence of HHE haplotypes was significantly higher in the HIV-positive group (in the entire cohort or in those who did not receive zidovudine prophylaxis) than in the HIV-negative group (table 2). Compared with those who lacked an HHE haplotype, possession of an HHE haplotype (i.e., 1 or 2 haplotypes) was associated with an ~2-fold increased risk of acquiring HIV-1 (table 3; HHE vs. non-HHE). The use of zidovudine prophylaxis was proportionally similar among all groups tested (P > .96; i.e., regardless of CCR5 haplogroup), which suggests that the increased susceptibility of acquiring HIV-1 in children who possess an HHE haplotype was independent of zidovudine use.

Because possession of an HHE haplotype appeared to be associated with a significantly increased risk of acquiring HIV-1, we next determined whether this effect was due solely to the combined presence of 2 HHE haplotypes or whether possession of 1 HHE haplotype (i.e., HHE heterozygosity) also influenced transmission. As shown in table 3, heterozygosity (HHE/HHE) or heterozygosity for HHE (HHE/non-HHE) was associated
with an increased risk of perinatal transmission, compared with those who lacked an HHE haplotype, with possession of 2 copies of HHE having a greater transmission-enhancing effect. Of the haplotype pairs that contained only 1 HHE and also had sufficient subject numbers for statistical analysis, 2 were associated with an increased risk of acquiring HIV-1 (table 4). Surprisingly, the pairing of CCR5 HHG*2, that is, the CCR5-Δ32-containing haplotype, with an HHE haplotype enhanced transmission, whereas the pairing of HHE with HHF*2 was associated with no transmission-modifying effect (table 4; and data not shown).

The aforementioned findings demonstrated that certain HHE-containing haplotype pairs are associated with transmission-enhancing effects relative to pairs that lack this haplotype. Table 5 shows that, of the 7 non–HHE-containing haplotype pairs that had sufficient numbers of subjects for statistical analyses, 4 were associated with a reduced risk of acquiring HIV-1 relative to those haplotype pairs that contained at least 1 HHE haplotype. In contrast to the pairing of the Δ32-containing haplotype HHG*2 with HHE, the combination of the HHG*2 with HHC was associated with the lowest risk of acquiring HIV-1 infection. HHG*1 is the genetic background on which the Δ32 mutation arose [59], and it is notable that the pairing of either this haplotype or HHG*2 with HHC afforded partial protection (table 5).

Haplotypes HHA/HHA (n = 3), HHF*1/HHF*1 (n = 1), HHG*1/HHG*1 (n = 1), HHG*2/HHG*2 (n = 1), HHB/HHE (n = 1), and HHHF*1/HHG*1 (n = 2) were found only among the uninfected children. In contrast, haplotype pairs HHF*1/HHG*2 (n = 2), HHD/HHF*2 (n = 3), and HHC/HHD (n = 3) were found only among the infected children (table 1). The small number of children who had these haplotype pairs limited the statistical power to detect an association between these haplotype pairs and the risk of transmission, which permitted only a qualitative analysis of their role in HIV transmission.

### Disease-modifying CCR5 genetic determinants

We next inquired whether the spectra of CCR5 haplotypes or haplotype pairs that influenced perinatal transmission were similar to those that altered the rate of disease progression in infected children. Analogous to the transmission-enhancing effects associated with possession of HHE, homozygosity or heterozygosity for HHE haplotypes was associated with an accelerated rate of progression to AIDS compared with children lacking an HHE haplotype (figure 2A, 2B). Homozygosity for HHE haplotypes was also associated with a more rapid progression to death (relative hazard [RH], 3.12; 95% confidence interval [CI], 1.0–9.93; P = .05; Kaplan-Meier curve not shown).

The transmission data in tables 3 and 4 suggested that a subset of HHE-containing haplotype pairs increased the risk of acquiring HIV-1. To determine whether a similar scenario was also operative for the HHE haplotype and its association with disease progression, we stratified into 4 groups the infected children who had an HHE haplotype, each group consisting of different haplotype combinations (figure 2C). A disease-accelerating effect was observed for the haplotype pairs HHE/HHE, HHC/HHE, and HHE/HHG*2 and for the pooled analysis of the haplotype combinations of HHE paired with HHA, HHD, HHF*1, or HHG*1 (figure 2C). In contrast, if an HHE haplotype was paired with a CCR2-64I-containing haplotype (HHF*2; figure 1), a haplotype associated with demonstrable disease retardation in this cohort (figure 3A; [51]), the disease-accelerating effects of the HHE haplotype were neutralized (figure 2C).

### Table 2. Frequency of CCR5 haplotypes associated with altered risk of human immunodeficiency virus (HIV) transmission in Argentinian children exposed perinatally to HIV.

<table>
<thead>
<tr>
<th>Haplotype or haplotype pair</th>
<th>All (adjusted for zidovudine prophylaxis)</th>
<th>No zidovudine prophylaxis</th>
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<td>HIV+</td>
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<tr>
<td>Haplotype</td>
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<td>453</td>
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<tr>
<td>Haplotype pair</td>
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<td>E/E</td>
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**NOTE.** Data are no. (%) of haplotypes or haplotype pairs. CI, 95% confidence interval; HIV−, HIV-negative children; HIV+, HIV-positive children; OR, odds ratio.

*Reference group.*
Compared with individuals who lack an HHF*2 haplotype, possession of HHF*2 was associated with a delay in progression to AIDS (figure 3A) and a trend toward delay in death (RH, 0.15; 95% CI, 0.02–1.11; P = .06; Kaplan-Meier curve not shown). Among all HHF*2-containing haplotype pairs, the most common were HHC/HHF*2 and HHE/HHF*2 (table 1). To examine the disease-modifying effect associated with these pairs, we stratified the patients who had an HHF*2 haplotype into 3 groups, each with a different haplotype in combination with HHF*2 (figure 3B). The maximum disease-retarding effect was observed for the haplotype pair HHC/HHF*2, compared with that in children lacking HHF*2 haplotypes (figure 3B). The clinical course was similar for those with the haplotype pair HHE/HHF*2 and for those lacking HHF*2 haplotypes (RH, 0.66; 95% CI, 0.35–1.26; P = .20; figure 3B).

By use of Cox proportional hazard models, possession of an HHA, HHD, HHG*1, or HHG*2 haplotype did not affect clinical outcome. The number of infected children who had the haplotype pair HHC/HHG*2 was too small for a separate time-to-event analysis. The use of zidovudine alone or in conjunction with additional antiretroviral agents was similar among children with different haplotype pairs (P > .10), suggesting that differences in therapy are an unlikely confounding factor to explain the disease-modifying effects associated with CCR5 haplotypes.

### Discussion

Two lines of evidence support our hypothesis. First, there is concordance between the CCR5 haplotype pairs that influence mother-to-child transmission and those that affect disease progression in infected children (figure 4A). Second, there is concordance between the CCR5 haplotype pairs that influence HIV-1 pathogenesis in children perinatally exposed to HIV-1 and those that we have shown previously to influence rate of HIV-1 disease progression in infected European American adults (figure 4B) [25]. For example, similar to the increased or reduced susceptibility of mother-to-child transmission of HIV-1 afforded by HHE/HHE or HHC/HHG*2, respectively, these haplotype pairs afforded maximal disease acceleration or retardation, respectively, in infected European American adults [25]. This overlap is remarkable because not only are the modes of transmission so different but also the AIDS manifestations in children are distinct from those in adults [40, 42].

In the context of the natural history of HIV-1 infection, this concordance is striking, because transmission and progression to an AIDS-defining illness are distinct aspects of HIV-1 pathogenesis and are temporally dissociated by a time interval that spans at least a few months [40]. In the context of the mechanisms of HIV-1 pathogenesis, this concordance suggests that, in addition to transmission, clinical disease outcome could also be mediated by events that occur during or shortly after initial virus exposure.

Critical host responses to HIV infection are likely to occur within hours to days of virus entry, but in humans it is challenging to study host-virus interactions during the very early stages of infection. However, studies in nonhuman primate models of HIV-1 infection [8–10, 61] implicate host rather than viral factors that act during the very early phases of infection as important determinants of the eventual plasma viremia set point. This is especially noteworthy, because the virologic set point level is highly predictive of subsequent clinical outcome not only in simian immunodeficiency virus–infected nonhuman

### Table 4. Transmission-enhancing CC chemokine receptor 5 haplotype pairs in Argentinian children exposed perinatally to HIV.

<table>
<thead>
<tr>
<th>Haplotype pair</th>
<th>All (adjusted for zidovudine prophylaxis)</th>
<th>No zidovudine prophylaxis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV⁺</td>
<td>HIV⁻</td>
</tr>
<tr>
<td>Non-E/Non-E*²</td>
<td>164</td>
<td>147</td>
</tr>
<tr>
<td>C/E</td>
<td>57</td>
<td>26</td>
</tr>
<tr>
<td>E/G*²</td>
<td>3</td>
<td>13</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of haplotype pairs. CI, 95% confidence interval; HIV⁺, HIV-positive children; HIV⁻, HIV-negative children; OR, odds ratio.

* Reference group.

### Table 5. Transmission-retarding CC chemokine receptor 5 haplotype pairs in Argentinian children exposed perinatally to human immunodeficiency virus (HIV).

<table>
<thead>
<tr>
<th>Haplotype pair</th>
<th>All (adjusted for zidovudine prophylaxis)</th>
<th>No zidovudine prophylaxis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV⁺</td>
<td>HIV⁻</td>
</tr>
<tr>
<td>E/E or E/non-E*²</td>
<td>138</td>
<td>200</td>
</tr>
<tr>
<td>A/C</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>C/C</td>
<td>44</td>
<td>35</td>
</tr>
<tr>
<td>C/G*¹</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>C/G*²</td>
<td>9</td>
<td>3</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of haplotype pairs. CI, 95% confidence interval; HIV⁺, HIV-positive children; HIV⁻, HIV-negative children; OR, odds ratio.

* Reference group.
Figure 2. Disease-accelerating effects associated with the CC chemokine receptor 5 (CCR5) HHE haplotype in infected Argentinean children. 

A. Kaplan-Meier (KM) curves for the development of AIDS in perinatally infected children who lacked (blue; reference group) or had (red) an HHE haplotype. The relative hazard (RH), 95% confidence interval (CI), and significance value (P) determined by use of the log-rank test for the comparison of the reference KM curve to the KM curve for children who had an HHE haplotype are shown. B. KM curves of the development of AIDS in perinatally infected children who had 1 (red) or 2 (green) HHE haplotypes. The reference group for survival analyses consisted of infected children who did not have an HHE haplotype (blue). The RH, 95% CI, and P value determined by use of the log-rank test for the comparison of the reference KM curve (blue) to the KM curve for children who are heterozygous or homozygous for the HHE haplotype are shown. C. KM curves of the development of AIDS in perinatally infected children with the following haplotype pairs: HHE/HHF*2 (light green); HHC/HHE (orange); HHE/HHE (red); and HHE/HHG*2 (dark green). Because of the small number of individuals who had these haplotype pairs, the disease course of individuals with the haplotype pairs that contained an HHE haplotype paired with HHA, HHD, HHF*1, or HHG*1 was combined and is shown as the black KM curve. The reference group is individuals who do not possess an HHE haplotype (blue). The RH, 95% CI, and P value determined by use of the log-rank test for the comparison of the reference KM curve (blue) to the KM curves for the indicated haplotype pairs are shown. The median time to AIDS is also shown for the color-coded KM curves. In panels A and C, “+” and “−” indicate the presence or absence of the indicated haplotype, respectively.

primates but also in HIV-infected humans [8, 9, 62–64]. Notably, in nonhuman primate models, these host factors exert their effects before the optimal development of specific antiviral immune responses, such as the effector phase of cytotoxic T lymphocyte activity or the production of antibodies to viral antigens [8, 9]. However, the identities of these host factors or the potential mechanism by which they influence these early events have, to date, remained elusive. Whether CCR5 could be such a factor is not known. However, we found that the CCR5 haplotype pairs that altered risk of transmission were also predictors of disease progression, suggesting that HIV-1 transmission and disease outcome may be mediated through interrelated CCR5-dependent mechanisms that are operative during the early stages of infection.

Measures of relative risk do not convey automatically the overall contribution of a risk factor to a disease in a particular population. From a public health perspective, in a given population, the risk that is attributable to a given CCR5 genetic factor is a function not only of the strength of its association but also of the prevalence of the factor in the population. For example, to date, 2 protective CCR5 genotypes, Δ32/Δ32 and Δ32/m303, have been discovered in individuals of European descent, and their combined prevalence in this population is ∼1% [1–5, 29, 65]. Thus, although the protective effect of these
2 genotypes is substantial, the attributable risk is small because of their low prevalence in the population. In this regard, the 4 CCR5 resistance factors that influenced perinatal transmission in Argentinean children (HHA/HHC, HHC/HHC, HHC/HHG*1, and HHC/HHG*2; table 5) are of significance from 2 perspectives. First, they provided partial protection in the context of a host that is highly exposed to HIV-1. Second, their combined prevalence is fairly high in this pediatric cohort (29.5% in HIV-negative vs. 18.5% in HIV-positive children), as well as in European Americans (~28%) and Hispanic Americans (~22%) [25], and they may potentially provide partial resistance in a fairly large proportion of individuals from these ethnic groups.

In terms of public health impact, the HHE/HHE haplotype pair could be of particular relevance. The prevalence of HHE in European American [25] and Argentinean populations is high, and a potentially important biologic role in promoting HIV-1 pathogenesis is indicated by it associations with adverse outcomes in 3 distinct settings: perinatal HIV-1 transmission and disease progression in infected children and in adults (figure 4). An analysis of the genotype-phenotype studies in other adult European American cohorts also indirectly indicates an important role for HHE/HHE in disease progression. The P1 and the 59029A alleles described by Martin et al. [28] and McDermott et al. [27], respectively, are essentially a combination of 3 haplotypes: HHE, HHG*1, and HHF*1 (figure 1B). Conceivably, the disease-accelerating effects of the P1/P1 or 59029A/59029A genotypes may be mediated, in part, by a subset of the P1 or 59029A haplotypes that have genotypic features similar to HHE.

The finding that the CCR5 haplotypes associated with altered rates of HIV-1 transmission or progression to disease overlap but are not identical in different populations should not be surprising for 3 reasons. First, the prevalence of different CCR5 haplotypes varies widely among different populations [25], and this may produce differences in disease susceptibility among populations (figure 4). This point is further reinforced by the recent findings of Kostrikis et al. [30] that homozygosity for a haplotype that corresponds to HHD is associated with increased risk for perinatal HIV-1 transmission in African American children. The HHD haplotype is found mainly in individuals of African origin [25], and because of the very low prevalence of this haplotype in our cohort, we were unable to determine its potential role in mother-child transmission. However, interestingly, of the 7 children who had an HHD haplotype, 6 were in the HIV-infected group (table 1). This finding, in conjunction with those of Kostrikis et al. [30] and our previous findings in HIV-infected African American adults (figure 4B; [25]) suggest that a subset of the HHD-containing haplotype pairs may play an important role in promoting HIV-1 pathogenesis.

Second, the same CCR5 haplotype or haplotype pair may be associated with different phenotypic effects among populations [25]. Third and most important, different combinations of a CCR5 haplotypes may be associated with very different phenotypes [25]. For example, it is generally believed that possession of a CCR5-Δ32-bearing haplotype (i.e., HHG*2) is associated with disease protection. However, our analysis of the haplotype pairs that contain an HHG*2 haplotype proved very different (figure 4A). The phenotype associated with HHG*2 depends highly on the other CCR5 haplotype, such that it can be associated with either enhanced (HHE/HHG*2) or reduced
arguing that and stigmatizing populations is not appropriate. To avoid this, we have used the term "Caucasian" as a placeholder, recognizing that it is a broad and diverse category with significant genetic diversity.

In the context of HIV-1 transmission and disease progression, it is clear that genetic factors play a significant role. For example, the CCR5 Δ32 mutation, found in individuals of European descent, confers a slower disease course in HIV-1 infection. However, the prevalence of this mutation varies significantly across different populations. In Argentinean children, the CCR5 Δ32 mutation was associated with the HHC/HHG*2 haplotype pair, whereas in African American adults, the maximum disease-retarding effect was associated with the HHA/HHF*2 haplotype pair. This indicates that the genetic context in which these factors operate can significantly influence disease outcomes.

In conclusion, understanding the complex interplay between genetics and disease progression is crucial for developing effective interventions. The role of genetic variation in HIV-1 transmission and disease progression highlights the need for continued research to elucidate the underlying mechanisms and to develop targeted therapies for different populations.

**Figure 4.** A. Concordance between the CC chemokine receptor 5 (CCR5) haplotype pairs that influence risk of transmission and disease progression in children exposed perinatally to human immunodeficiency virus type 1 (HIV-1). B. For comparative purposes, CCR5 haplotype pairs associated with the maximum disease-retarding or -accelerating effects that were determined previously [25] in infected European American and African American adults are shown. The importance of understanding the overall genetic context in which a CCR5 haplotype is found is further illustrated by our findings related to the CCR2-64I-containing haplotype (HHF*2; figure 1) in HIV-1 transmission and disease. In Argentinean children, the maximum disease-retarding effect was associated with the HHC/HHF*2 haplotype pair, whereas in African American adults the maximum disease-retarding effect was associated with the HHA/HHF*2 haplotype pair [25]. In contrast, a significant disease-modifying effect for the CCR2-64I-containing haplotype was not found in the seropositive European Americans that we studied previously [25, 26]. Notably, the HHA haplotype is the ancestral CCR5 haplotype [59], and both HHA and HHF*2 are found at a higher frequency in individuals of African or Hispanic descent, compared with those of European descent [25]. The [64I.P1. +[I.+P4. +] genotype described by Martin et al. [28] has genotypic features similar to those of HHF/HHF*2 and was among the genotypes that were associated with a slower disease course in an analysis of individuals broadly classified as Caucasians [25, 28, 59].

(HHC/HHG*2) susceptibility to transmission or disease progression. Because of the genetic heterogeneity of populations that are often called “Caucasian,” the prevalence of HHC, HHE, and HHG*2 may vary substantially from cohort to cohort. For example, individuals in Europe are broadly classified as being Caucasian; however, the prevalence of the CCR5-Δ32 haplotype varies significantly in countries in the northern (e.g., Finland) or southern (e.g., Italy) parts of Europe [66]. This will affect the prevalence of HHC/HHG*2 and HHE/HHG*2 among all HHG*2-bearing haplotype pairs in a cohort. This may explain why, in previous analyses restricted to CCR5-Δ32 heterozygotes, no association or a discordant association was found between HHG*2 and mother-to-child transmission of HIV-1 [43, 46, 47, 50, 52] and sexual transmission in Caucasian individuals [3–5, 17], respectively.

Pinpointing the precise mechanisms that underlie the CCR5 genotype-HIV transmission and/or disease phenotype associations remains a challenging task. Mechanistically, distinct CCR5 genetic factors may determine a “CCR5 set point” for its expression levels in HIV-1 target cells. This threshold or set point for CCR5 cell surface density, either in the basal state or after agonist/HIV-induced internalization [67, 68] and subsequent receptor cycling [69, 70], could potentially affect the intrinsic susceptibility of target cells and thereby influence the extent of early viral replication. In this context, CCR5 surface density correlates with cell infectability in vitro [6, 71], and HIV RNA plasma levels correlate with peripheral blood CD4⁺ T cell surface CCR5 density [72]. In addition, given the emerging role of the chemokine system in mediating host immune responses [73], it is conceivable that CCR5 genetic variation may influence the robustness of the innate immune response or the qualitative nature of the immune response to HIV infection [74]. The molecular mechanisms by which CCR5 polymorphisms or haplotypes influence gene expression also are emerging, and our early studies indicate that polymorphisms in the cis-regulatory regions of CCR5 are associated with differential haplotype-specific transcriptional activity and cell type–specific differential nuclear factor binding [59]. Notably, there is strong precedence linking genetic variation in the cis-regulatory regions of genes and pathogenesis of infectious diseases [75, 76].

Several limitations of this study merit consideration. First, our cohort comprises children exposed perinatally to HIV-1 infection, and thus the results might not apply to other risk groups. For example, failure to find complete identity between the haplotype pairs that influence pathogenesis in Argentinean children and European American adults could reflect that CCR5 haplotypes have different effects on vertically (mother-child) versus horizontally transmitted HIV and/or disease progression in children versus adults. Alternatively, this difference might reflect genetic differences in these 2 populations. Second, we determined the CCR5 haplotype pairs of the children only. Clearly, the mother’s CCR5 genetic constitution is also likely to influence her own disease status, which, in turn, could potentially make her more or less likely to transmit virus, as has been shown recently for the mutation in stromal derived factor-1, the ligand for the coreceptor CXCR4 [34]. In this study, we found several examples in which the same CCR5 haplotype pair that influenced HIV pathogenesis in the perinatal setting also influenced outcome in a completely distinct HIV setting (figure 4). For example, HHC/HHG*2 was associated with decreased mother-to-child transmission and slowed disease course in the infected European Americans that we studied [25]. An analysis of the work of Martin et al. [28] suggests that HHC/HHG*2 is also protective in other European American cohorts. The +.P4.+ and +.P1.Δ32 alleles described by Martin et al.
have genotypic features that correspond to the haplotypes found in CCR5 human haplogroups C and G*2, respectively [25, 28, 59]. Similarly, as discussed above, homozygosity for HHE also influenced pathogenesis in distinct clinical settings. Taken together, although we cannot accurately assess the contribution of maternal factors in a child’s susceptibility to acquire HIV-1, the identification of the same CCR5 haplotype pairs in different contexts but similar ethnicities (European origin) suggest that a child’s CCR5 genetic makeup also is likely to play an important role. Third, our studies provide evidence in support for our hypothesis, but they do not provide definitive proof that CCR5 genetic determinants influence the early host events that are initiated after infection.

Collectively, our findings have several conceptual and practical implications. First, in each population, a distinct spectrum of CCR5 haplotype pairs is associated with altered susceptibility to HIV-1 infection. Within this spectrum, a subset is associated with maximal protection, whereas another subset is associated with adverse effects (figure 4). Thus, differences between cohorts in the transmission- and/or disease-modifying effects of CCR5 haplotypes are probably due to ethnicity-dependent differences in the prevalence of these haplotypes, which suggest that CCR5 genotype–HIV phenotype association data derived from one population may not be generalizable to other populations. In a broader context, this interpopulation heterogeneity of CCR5 resistance or susceptibility haplotypes is also of relevance to the increasing numbers of studies aimed at understanding the association between genetic variation in CCR5 and other disease states, such as rheumatoid arthritis [77–80], sarcoidosis [81], multiple sclerosis [82], or asthma [83] in different ethnic populations.

Second, CCR5 genetic factors are a powerful determinant of mother-to-child transmission and disease progression. Our findings also suggest that these genetic factors may influence the course of HIV-1 pathogenesis soon after infection has occurred, reinforcing the growing notion that the window of opportunity within which potentially protective interventions can be used to alter host-virus equilibrium probably is very limited [84]. From a practical standpoint, any alteration of the natural host-virus equilibrium might be possible only through prior vaccine intervention or antiviral therapy applied within a short period after HIV-1 exposure. Third, our findings emphasize the importance of understanding haplotype-haplotype interactions in studies that attempt to understand the link between host genotype and HIV-1 transmission and/or disease phenotype.

Acknowledgments

We thank R. B. Kasinath and A. Diehl (University of Texas Health Science Center at San Antonio), J. Allan (Southwest Foundation for Biomedical Research), and B. Cherniak for advice; S. Cabrera, R. Geervarghese, A. Rivera, X.-H. Cao, J. Barnes, and C. Hensler (University of Texas Health Science Center at San Antonio) for superb technical assistance; and A. S. Ahuja for forbearance. We thank the children and parents who made this study possible and the 3 anonymous reviewers of this paper for their valuable comments.

References


