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Protective Effect of CCR2-64I and Not of CCR5-Δ32 and SDF1-3′A in Pediatric HIV-1 Infection

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Summary: The effects of chemokine and chemokine receptor genetic polymorphisms such as stromal derived factor 1 (SDF1-3′A), CCR2-64I, and CCR5-Δ32 associated with HIV-1 transmission and/or rate of disease progression in infected study subjects remain highly controversial and have been analyzed primarily only in adults. We have investigated whether these polymorphisms may provide similar beneficial effects in children exposed to HIV-1 perinatally. The prevalence of CCR2-64I allele was significantly increased ($p = .03$) and the CCR2-64I genotype distribution was not in Hardy-Weinberg equilibrium, among HIV-1–exposed uninfected infants. Moreover, in the HIV-1–infected group, a delay to AIDS progression was observed among carriers of CCR2-64I allele. This is the first report that suggests a protective role of CCR2-64I allele in mother-to-infant HIV-1 transmission and documents a delay in disease progression, after the child has been infected with HIV-1. However, SDF1-3′A and CCR5-Δ32 alleles did not modify the rate of HIV-1 transmission or disease progression in HIV-1–infected children. **Key Words:** HIV-1—CCR5—CCR2—SDF1—Chemokine receptors—Vertical transmission—Pediatric AIDS.

Host genetic factors may modify susceptibility to HIV-1 infection and affect the clinical outcome to AIDS and/or death in infected study subjects. In addition to the CD4 molecule, HIV-1 requires cellular cofactors to infect target cells. HIV-1 T-cell–tropic (T-tropic) isolates use as coreceptors CXC-chemokine receptors, specifically CXCR4 (1,2), whereas macrophage-tropic (M-tropic) strains are engaged with CC-chemokine receptors (3,4), mainly CCR5 but also with minor coreceptors such as CCR2b and CCR3 (5,6). Moreover, there are HIV-1 dual-tropic variants that can bind to both types of chemokine receptors (6). Polymorphisms of some chemokines and chemokine receptors may modify HIV-1 transmission and disease progression (7–10). The first one identified was an homozygous 32-bp deletion in the coding region of the CCR5 gene that hinders the cellular expression of the HIV-1 coreceptor and provides a strong, but not absolute, resistance to M-tropic viral infection (11–18). However, once HIV-1 switches to other coreceptors, such as CCR1 to CCR4 and/or CXCR4, the homozygous CCR5-Δ32 deletion can no longer exert any protection (19,20). The presence of a single copy of CCR5-Δ32 does not seem to protect against HIV-1 transmission but there is an unequivocal protective effect for heterozygotes with CCR5-Δ32 compared with CCR5 wild-type infected adults in disease progression (7,10,12,21–24). Thereafter, a point mutation in the CCR2b gene (CCR2-64I) has been observed to prolong AIDS-free survival from 2 to 4 years in HIV-1 seroconvertors (9,25,26). Winkler et al. (27) described the first polymorphism of a chemokine gene, the stromal derived factor 1 (SDF1), that can improve the clinical outcome of HIV-1 infection. The mutation was identified as a change from guanine to adenine in the 3′ untranslated region (3′UTR) of the SDF1β gene transcript (SDF1-3′A).

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In HIV-1 infection, the protective effect that may confer the mutated alleles of the three genes already mentioned have not been demonstrated in all the cohorts studied (10,25). Most studies have been carried out in adults, and the inconsistent findings derived from these studies can be attributed to multiple factors. The difficulty in establishing the timing of the viral infection in adults, racial background, route of infection, sample size, and variation in prospective or retrospective studies represent some factors that are considered to produce a bias in results (25,28). The perinatally HIV-1–infected cohort, with less complexity and variability, and with a more precise timing of the viral infection, may be considered a much better model to study the influence of host genetic factors on HIV-1 disease progression. Studies on HIV-1–infected infants are particularly scarce, and only analysis of CCR5 genetic polymorphism have been reported with discordant results (29–33).

The aim of the study was to investigate the role of CCR2-64I and SDF1-3′A alleles in HIV-1 vertical transmission and, together with the CCR5-Δ32 allele, their impact in perinatally HIV-1–infected children.

METHODS

Patients

Informed written consent was obtained from the parents or legal guardians for the children who were included in this study. The study included stored cell lysates obtained from 886 children exposed to HIV-1 perinatally who had been born to HIV-1–seropositive mothers between 1986 and 1998, who were treated at the Hospital de Pediatría "J. P. Garrahan" of Buenos Aires, Argentina. Of them, 449 were uninfected and 437 had been infected with HIV-1 by vertical transmission. HIV-1 infection status, AIDS definition, and stage of immune suppression were established according to the 1994 criteria of the U.S. Centers for Disease Control and Prevention (CDC) classification for children (34). According to the AIDS Clinical Trials Group (ACTG) 076 protocol (35), zidovudine (ZDV) prophylaxis of mother-infant pairs (a three-part regimen) was given to 220 children (of whom 192 were uninfected and 28 infected), and partially (mother or child) given to 27 (6 uninfected and 21 infected) children. In total, 554 children (194 uninfected and 360 infected) did not receive ZDV, whereas in 85 (57 uninfected and 28 infected) the information was unavailable because the data had not been entered in medical records and we could not thus obtain the information retrospectively. Since 1992, all infected children have received antiretroviral therapy following the recommended guidelines (36,37) in use before the addition of protease inhibitor therapy. Survival analysis of HIV-1–infected children was restricted to 382 infants in whom clinical follow-up was recorded (382 were analyzed for CCR2, 381 for CCR5, and 378 for SDF1). The differences in the number of patients analyzed for each polymorphism derived both from the lack of polymerase chain reaction (PCR) amplification, as well as a dearth of samples.

Genotyping Analysis

These PCR assays were performed directly on cell lysates. The CCR5 and CCR5-Δ32 alleles were amplified by PCR with specific primers (29). The expected PCR products of 184 bp corresponding to the wild-type allele, and 152 bp corresponding to the Δ32 allele were separated on 2.5% agarose gels. CCR2 genotypes were determined with a PCR-restriction fragment length polymorphism (RFLP) assay using the method reported by Smith et al. (9). The sense primer contains a mismatch that introduces a *BSA B*I restriction endonuclease site in combination with the C-T transition that generates the 64I mutation. Digestion of the 128-bp amplified fragment yielded fragments of 110 and 18 bp when the CCR2-64I polymorphism was present. The digested products were run on 4% 3:1 high-resolution blend agarose gels (AMRESCO, Inc., U.S.A.). The SDF1-3′A polymorphism was determined with a PCR-RFLP assay amplifying a fragment from the 3′UTR of the gene using the primers reported by Winkler et al. (27). The PCR product was digested with *MspI* and the resulting fragments of 302 bp for the SDF1-3′A allele, and two fragments of 202 and 100 bp for the SDF1 wild-type allele were identified on 1.5% agarose gels.

Statistical Analysis

Allele frequencies were calculated and the fit to Hardy-Weinberg equilibrium among the studied population was evaluated with the χ^2 test. Contingency tables were performed using either χ^2 test or Fisher's exact test. The Kaplan-Meier method was used to estimate disease progression, survival time, and probability of develop severe immune deficiency, and the differences were tested by the log-rank test. Relative hazards were calculated by the Cox proportional hazard analysis. Patients who did not reach an endpoint were censored at the time of the last follow-up.

RESULTS

CCR2, CCR5, and SDF1 Genotypes in HIV-1 Vertical Transmission

To determine the frequency of the CCR2-64I, CCR5-Δ32, and SDF1-3′A alleles in the Argentine population, we studied randomly selected HIV-1–seronegative blood donors, including some previously reported CCR5 polymorphism data (Table 1) (29). The CCR2-64I, CCR5-Δ32 and, SDF1-3′A allelic frequencies were 0.1289, 0.0463 and, 0.2422, respectively, and their genotype distribution conformed to expectations of Hardy-Weinberg equilibrium ($p > .1$). The CCR2-64I, CCR5-Δ32, and SDF1-3′A alleles are present in our study population with a genotype distribution slightly different than that seen in Caucasian populations previously reported (9, 27,38).

We further investigated the role of CCR2-64I and SDF1-3′A alleles in HIV-1 vertical transmission, extending our initial studies of CCR5-Δ32 allele. We analyzed a pediatric cohort of 886 HIV-1–exposed children, of whom 437 were HIV-1–infected and 449 were uninfected (Table 1). CCR2-64I genotype distribution between HIV-1–infected and uninfected children did not differ significantly using Fisher's exact test ($p = .11$). However, this distribution was not in equilibrium as predicted by Hardy-Weinberg ($p < .01$). The CCR2-64I al-

TABLE 1. *CCR2, CCR5, and SDF1 genotype distribution in perinatally HIV-1–exposed children and normal blood donors*

Genotypes	HIV-1–uninfected *n* (%)	HIV-1–infected *n* (%)	HIV-1–seronegative blood donors *n* (%)
CCR2+/+	314 (70.40)	329 (75.29)	74 (76.29)
CCR2+/64I	108 (24.22)	95 (21.74)	21 (21.65)
CCR264I/64I	24 (5.38)	13 (2.97)	2 (2.06)
Total	446	437	97
p Value (HWE)	<.01	>.1	>.1
CCR5+/+	415 (92.43)	397 (91.69)	99 (91.67)
CCR5+/Δ32	33 (7.35)	36 (8.31)	8 (7.40)
CCR5Δ32/Δ32	1 (0.22)	0 (0)	1 (0.93)
Total	449	433	108
p Value (HWE)	>.1	>.1	>.1
SDF1+/+	270 (61.50)	286 (66.51)	37 (57.81)
SDF1+/3′A	153 (34.86)	131 (30.47)	23 (35.94)
SDF13′A/3′A	16 (3.64)	13 (3.02)	4 (6.25)
Total	439	430	64
p Value (HWE)	>.1	>.1	>.1

Numbers in parenthesis indicate the genotype frequency.
HWE, Hardy-Weinberg equilibrium.

lelic frequency was 0.1749 in the uninfected children ($n = 132$) and 0.1384 in the infected ones ($n = 108$), with a significant increase in the prevalence of the CCR2-mutated allele among the HIV-1–exposed uninfected infants ($p = .03$, Fisher's exact test). Overall these results suggest a protective effect of the CCR2-64I in mother-to-child HIV-1 transmission. Since 1996, the ACTG 076 protocol (35) has been used in 247 infants, including 27 who received a partial regimen of ZDV.

Therefore, to determine whether ZDV prophylactic therapy may have affected the genotype distribution of CCR2-64I in the total study population of children born to HIV-1–infected mothers, 82 were excluded because some information was unavailable and we then subdivided the remaining 801 according whether they had been treated according to the protocol (Table 2). No significant shift of the CCR2-64I allelic frequency was observed either in HIV-1–exposed uninfected infants, or in the infected group between those with and without receiving ZDV.

Within the same cohort of children exposed to HIV-1 infection, no significant differences were found in CCR5-Δ32 and SDF1-3′A genotype distributions between HIV-1–infected and uninfected children, indicating that these alleles do not modify HIV-1 perinatal transmission.

Role of CCR2-64I in HIV-1 Disease Progression

Kaplan-Meier analysis of time to AIDS showed that children carriers of a CCR2-64I allele ($n = 93$) progressed to AIDS significantly slower than CCR2 wild-type homozygous ($n = 289$; $p = .007$, log rank test; Fig. 1*A*). The benefit was indicated by a median AIDS-free survival time of 87 months in CCR2-64I carriers compared with 38 months in CCR2 wild-type homozygotes. The CCR2-64I allele also was a significant variable for progression to AIDS based on Cox proportional hazard analysis (Table 3). Of 27 patients who died during the study, only 1 carried a CCR2-64I allele. Analysis of time to death showed a statistically significant difference between CCR2-64I carriers and CCR2 wild-type homozygotes ($p = .01$, log rank test; Fig. 1*B*; Table 3). These data indicate that CCR2-64I allele improves the clinical outcome of HIV-1–infected children postponing progression to AIDS and death.

Role of CCR5-Δ32 in HIV-1 Disease Progression

From Kaplan-Meier plots (Fig. 1*C*; Table 3), it was estimated that children heterozygous for the CCR5-Δ32

TABLE 2. *Relation between CCR2 genotype and ACTG-076 protocol*

	Without 076 protocol	With 076 protocol[a]
HIV-1–uninfected		
$CCR2^{+/+}$	137 (70.62)	142 (71.72)
$CCR2^{+}$/64I	48 (24.74)	45 (22.73)
CCR2 64I/64I	9 (4.64)	11 (5.55)
Total	194	198
HIV-1–infected		
$CCR2^{+/+}$	269 (74.72)	37 (75.51)
$CCR2^{+}$/64I	78 (21.67)	12 (24.49)
CCR2 64I/64I	13 (3.61)	0
Total	360	49

Numbers in parenthesis indicate the genotype frequency.
[a] Including three-part or partial zidovudine regimen.
ACTG, AIDS Clinical Trials Group.

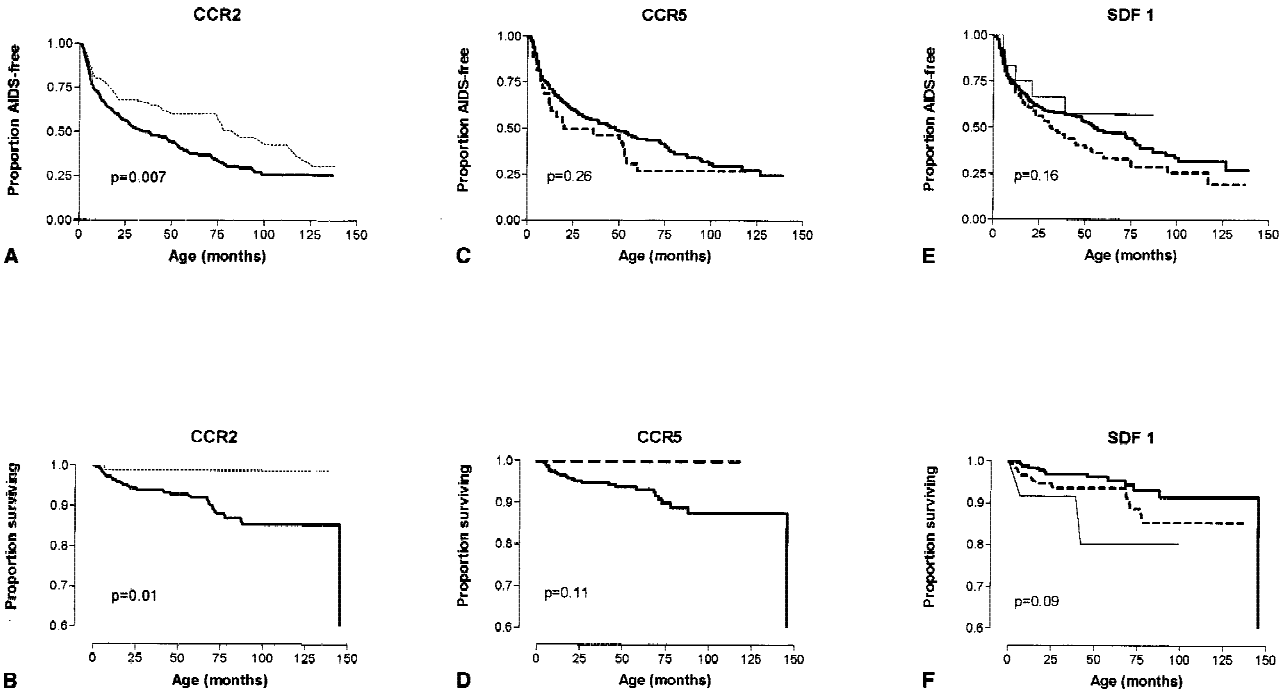

FIG. 1. Effect of CCR2, CCR5, and SDF1 genotypes on disease progression. Kaplan-Meier plots for time in months after birth to AIDS (**A, C, E**) and death (**B, D, F**) in HIV-1–infected children. Wild-type homozygotes are indicated by *thick lines,* heterozygotes by *thick dashed lines,* and mutated homozygotes by *thin lines.* CCR2-64I heterozygotes and homozygotes were pooled and indicated by *thin dashed lines.* Log-rank test *p* values from Kaplan-Meier analyses are shown.

allele ($n = 32$) developed AIDS at a median age of 20 months, not differing with the 48 months of the children with the CCR5 wild-type homozygous allele ($n = 349$). None of the 27 children who died during the study carried the CCR5-Δ32 allele. However, no significant difference in survival time was observed (Fig. 1*D*; Table 3). Our data indicate that CCR5-Δ32 does not have a protective effect against pediatric AIDS progression and death.

Role of SDF1-3′A in HIV-1 Disease Progression

Analysis of disease progression was carried out in 378 HIV-1–infected children, of whom 248 were wild-type homozygotes for the SDF1 gene, 118 carried an heterozygous genotype, and 12 were homozygous for the SDF1-3′A mutation.

Kaplan-Meier plots for time to AIDS and analysis by Cox proportional hazard regression (Fig. 1*E*; Table 3) showed no significant difference between the three groups. The SDF1-3′A was present in 12 of 25 children who died during the study, with a rate of survival time not reaching significance (Fig. 1*F*; Table 3). Thus, SDF1-3′A polymorphism does not seem to protect against the onset of AIDS and/or death in infected infants.

Role of CCR2, CCR5, and SDF1 Polymorphisms in Severe Immune Suppression

The probability of developing stage 3 immune suppression (34) related to the polymorphisms in the CCR2, CCR5, and SDF1 genes in HIV-1–infected children was

TABLE 3. *Cox proportional hazard model of the data shown in Figure 1*

	CCR2 (+/+) vs. (+/64I) or (64I/64I)			CCR5 (+/+) vs. (+/Δ32)			SDF1 (+/+) vs. (+/3′A) or (3′A/3′A)		
Endpoints	*n*/events	*p* Value	RH	*n*/events	*p* Value	RH	*n*/events	*p* Value	RH
AIDS	382/214	0.01	0.68	381/214	0.37	1.22	378/210	0.71	1.1
Death	382/27	0.04	0.13	381/27	1.00	0.00	378/25	0.08	1.7

RH, relative hazard.

analyzed. Data for CD4 T-cell counts were recorded in 352 infected children. None of the three polymorphisms studied showed significant differences in the rate of severe immune suppression by Kaplan-Meier analysis (data not shown). However, the CCR2-64I genotype tended to delay early progression to severe immune deficiency with a median of 110 months much slower than the 79 months of the homozygous wild-type. Conversely, the CCR5-Δ32 genotype tended to accelerate immune suppression for a median of 65 months versus 85 months in the homozygous wild-type. We also analyzed distribution by gender and determined that it did not affect the rate of AIDS progression and death in HIV-1–infected infants (data not shown).

DISCUSSION

This is the first study that suggests a protective effect of CCR2-64I allele in HIV-1 infection by vertical transmission, with a clear delay in progression to AIDS and death in perinatally HIV-1–infected children. The CCR5-Δ32 and SDF1-3′A polymorphisms do not modify either mother-to-child HIV-1 transmission or clinical outcome to AIDS.

The CCR2-64I protective effect was suggested by an overrepresentation of the mutated allele with a Hardy-Weinberg disequilibrium among the uninfected children born to HIV-1–infected mothers. The ongoing selection for the CCR2-64I allele persisted among the uninfected children, regardless of whether the mother-infant pairs received ZDV. Because the number of HIV-1–infected children who received the ACTG 076 protocol was limited, further studies are required to confirm the protective properties of CCR2-64I despite of the effect of antiretroviral drugs. However, CCR2-64I polymorphism did not seem to prevent HIV-1 sexual or parenteral transmission (9,26). The small number of uninfected subjects compared with the much higher number of those who had been exposed to HIV-1 may explain the discrepancy with our results.

In accord with studies done in HIV-1–infected adults, mainly in seroconvertors (9,24–26), we also found that the CCR2-64I allele not only prolongs the AIDS-free period but also the survival time to death in HIV-1–infected children, with a tendency to delay progression to the severely immunosuppressed state. The fact that CCR2 is a sporadic coreceptor for HIV-1 M-tropic strains and that CCR2-64I does not seem to affect CCR2 expression, makes it difficult to explain the protective effect observed. It has been attributed on one hand by altering the CCR5 surface expression due to a linkage disequilibrium with a polymorphism in the CCR5 regulatory region (25,26) although in vitro studies no association was found (39). Conversely, the CCR2-64I effect might be secondary to other unknown protective polymorphisms (9). The beneficial CCR2-64I effect in HIV-1 infection remains puzzling and unresolved.

The enlargement of patient population studied (from 328 in a former study [29] to 882), allowed us to confirm that CCR5-Δ32 heterozygosity does not confer protection in HIV-1 vertical transmission, as reported by others (30–33).

In contradistinction to the conclusions in the report of Misrahi et al. (30), we did not find any significant association between CCR5-Δ32 allele and disease progression or immune suppression at different stages of the viral infection in HIV-1–infected infants. The discrepancy between both studies could be related to their having analyzed different ethnic groups. Our cohort was mainly composed of Spanish and Italian descendants with lack of African and little Amerindian admixtures, with an allele frequency of 0.042 significantly lower ($p = .005$) than the 0.085 of the French Pediatric HIV Cohort (30) that included 152 non-African children. Moreover, our study cohort was seen at a single pediatric center, whereas the French cohort derived data from 52 different medical centers. Furthermore, although not significant, the survival curves of progression to AIDS and to severe immune suppression tended to be more pronounced in children carrying the heterozygous CCR5-Δ32 genotype than the homozygous wild-type, as observed by Esposito et al. (32). The state of immunocompetence at the time of HIV-1 infection, still immature in infants compared with the fully developed immunity found in adults, may contribute to the different effect of CCR5-Δ32 observed in children and adults.

The effects of SDF1-3′A mutation in the clinical outcome of HIV-1–infected adults have been highly discrepant. Although Winkler et al. (27) first reported an association of SDF1-3′A homozygosity with a delay in disease progression, van Rij et al. (40) observed a more rapid progression to AIDS followed by an elongated survival time after AIDS diagnosis. Conversely, Mummidi et al. (25) found an accelerated progression to death. In our pediatric cohort, we did not find either any association with SDF1-3′A by vertical transmission or in the clinical course of HIV-1 infection. Therefore, the effect of SDF1-3′A allele remains to be defined.

Host genetic polymorphisms are in part contributing to understand HIV-1 pathogenesis. Variation in HIV-1 transmission and/or disease progression may be the result of a complex combination of host genetic factors. Therefore, the study of a single gene polymorphism may provide only a partial view. Metaanalysis including mul-

tigenetic influences may contribute to reach more accurate and comprehensive conclusions.

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