Original Contributions

CCR5 Chemokine Receptor Variant in HIV-1 Mother-to-Child Transmission and Disease Progression in Children

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Context.—Studies suggest that adults with the CCR5Δ32 deletion are less likely to become infected with the human immunodeficiency virus (HIV) and to develop HIV-related disease progression, but the effect of the mutation in children is not known.

Objective.—To study the effect of the CCR5 chemokine receptor mutant allele on mother-to-child transmission of HIV type 1 (HIV-1) and subsequent disease progression in infected children.

Design.—Multicenter, prospective study of infants born to mothers seropositive for HIV-1.

Setting.—A total of 52 medical centers participating in the French Pediatric HIV Cohort studies.

Participants.—The CCR5Δ32 deletion was studied in 512 non-African children, born between 1983 and 1996 to HIV-1–infected mothers. Among them, 276 children were infected and 236 were not.

Main Outcome Measures.—HIV-1 infection status and, in infected children followed up since birth, incidence of category B and C disease events and severe immunosuppression as defined in the new pediatric Centers for Disease Control and Prevention (CDC) classification, according to CCR5 genotype.

Results.—The 32–base pair deleted allele was detected at a frequency of 0.05. Only 1 infant, not infected by HIV-1, was homozygous for the Δ32 deletion. The 49 heterozygous children (9.6% of the total; 95% confidence interval [CI], 7.1-12.2) were equally distributed into the infected (9.8%) and uninfected (9.3%) groups. The incidence of stage C symptoms in heterozygous infected children was 9% at 36 months vs 28% in children homozygous for the normal allele (P<.004). The proportion of children at 8 years old with no stage B or C symptoms was 49% for heterozygous children and 11% for children homozygous for the normal allele (P<.003). The progression of severe immune deficiency (CD4 <15%, CDC stage 3) was also significantly different between the 2 groups (P<.001).

Conclusions.—Heterozygosity for the CCR5Δ32 deletion does not protect children from infection by the maternal virus but substantially reduces the progression of the disease in HIV-1–infected children.

STUDIES IN adults have shown that homozygosity for a deletion of 32 base pairs (bp) (Δ32) in the CCR5 gene is associated with substantial—but not absolute—resistance to infection with the human immunodeficiency virus type 1 (HIV-1).1-3 Findings concerning the protection conferred by heterozygosity are conflicting.4-6 In terms of disease progression, the beneficial impact of heterozygosity is clear in some reports4,5,12 or modest and limited to biological variables in others6,11 or not confirmed.13

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The effects of this mutated allele on mother-to-child transmission of HIV-1 and subsequent disease progression in children are unknown. In the absence of antiviral prophylactic treatment, the rate of mother-to-child transmission is about 20%, depending on the rate of viral replication, the immune status of the mother, and various obstetrical factors.14-19 The mechanisms of transmission remain unknown. Possibly, free or intracellular virus penetrates the fetal blood compartment via the placenta. Alternatively, virus present in the amniotic fluid or vagina may infect the fetus via the mucosa, digestive tract, or skin. Data suggesting a genotype specificity of the virus transmitted to the child20 have not been confirmed.21 Furthermore, there are no accurate data available about either the phenotype or the tropism of the virus initially infecting the child, or whether these characteristics are different for blood and mucosal routes of transmission. Despite studies on HLA genotyping,22 the contribu-
tions of genetic factors to either mother-to-child transmission or severity of disease in infected children have not been established.

METHODS

The group studied was composed of 512 non-African children born between 1983 and 1996 to HIV-1-infected mothers followed up in 52 Paris area hospitals and for whom the virological analysis was performed at Necker Enfants Malades hospital. Children with 1 or 2 parents of sub-Saharan African (n=153) or Caribbean (n=31) origin were excluded, because of the expected absence or lower frequency of the mutated allele in these populations. Of this group, 343 were enrolled at birth in the previously described French Pediatric HIV Cohort.14 Other study group subjects were identified as infected with HIV at various ages and regularly followed up in the Necker Enfants Malades hospital. Ultimately, 276 infected children and 236 uninfected children, matched for year of birth, were studied. Most of the children (>85%) were born before 1994, when the program for prevention of mother-to-child transmission using zidovudine was implemented.

The diagnosis of HIV infection for the children was based either on HIV antibody status or on the results of 2 virologic tests (culture or polymerase chain reaction [PCR]), at least 1 of which was done after 3 months as previously described.19 The recommendations of the 1994 Centers for Disease Control and Prevention (CDC) classification of HIV-related disease events were used for clinical and biological classification of infected children.20 In summary, class C events include opportunistic infections, severe and repeated bacterial infections, specific encephalopathy, wasting syndrome, lymphoma, and neoplasia. Class B includes all other symptoms related to HIV not included in class C (such as lymphoid interstitial pneumonitis, nephropathy, cardiomyopathy). Severe immunosuppression as defined by class 3 immune deficiency (<15% CD4) was also assessed.21

The French Pediatric HIV Cohort Study received approval from the Hôpital Necker ethics committee and the Commission Nationale de l’Informatique et des Libertés (a computer database watchdog group). For study subjects not in the cohort, informed consent was obtained from the family. For children who had died at the time of the analysis, tests were performed on frozen samples according to French law 94 548 (July 1994).

CCR5 Genotyping

Polymerase chain reaction amplification of CCR5 alleles was performed on genomic DNA prepared from frozen peripheral blood mononuclear cells by standard methods. Two primers encompassing the 32-bp deletion found in CCR5Δ32 were used:

| Forward primer: | TTCATTACACCTGCAGCTCTCATTTTC |
| Reverse primer: | CTCACAGGCTGTGCCTCTTCTTC |

yielding, respectively, a 184-bp fragment for the wild-type (WT) allele and a 152-bp fragment for the mutated allele.

The primers were labeled with a fluorochrome, and the Pharmacia Taq polymerase (Biotech, Uppsala, Sweden) was used for PCR amplification. After a denaturation step of 95°C for 10 minutes, 30 cycles of PCR were performed as follows: a denaturation step for 1 minute at 93°C, a hybridization step for 1 minute at 54°C, and an extension step for 6 minutes at 72°C. A final extension step of 10 minutes at 72°C was then performed. The labeled amplified fragments were resolved by capillary electrophoresis and were analyzed using an ABI PRISM 310 genetic analyzer (Applied Biosystems, Foster City, Calif). For 4 samples (1 WT/WT, 2 WT/Δ32, and 1 Δ32/Δ32), the sequence of the amplified product was also directly determined (manually sequenced) to ascertain the nature of the WT and mutated CCR5 alleles and confirmed the results from automated sequencing. A 373 A sequence (Applied Biosystem, Foster City, Calif) was used.

Statistical Analysis

Proportions were compared using the χ² test or Fisher exact test for expected values below 5. When necessary, Geylig scientific tables were used to determine the 95% confidence interval (CI). The Kaplan-Meier method was used to establish the cumulative incidence of the events under consideration. They were compared by using log-rank tests. SAS software (SAS Institute, Cary, NC) was used for statistical analysis.

RESULTS

CCR5 Genotype Distribution

The CCR5 genotype of 512 children born to HIV-1-seropositive mothers was determined; 276 were infected by HIV-1 and 236 were uninfected. Allele frequencies of the whole study group were 95% for the WT allele and 5% for the mutated allele. One child was homozygous for the CCR5Δ32 deletion. A total of 49 children were heterozygous (9.6%; 95% CI, 7.1-12.2). Genotype frequencies were not significantly different from what would be expected according to the Hardy-Weinberg law of equilibrium22 (which indicates that it is unlikely selection has occurred). For 512 patients with the WT/WT genotype: observed, 462; expected, 462.3. For WT/Δ32: observed, 49; expected, 48.5. For Δ32/Δ32: observed, 1; expected, 1.3. The homozygous child was not infected by HIV-1, and thus, the frequency of homozygosity among the uninfected children was 0.4% (95% CI, 0.08-2.8).

CCR5 Genotype and Mother-to-Child Transmission

The distribution of the heterozygotes into the infected (27/276, 9.8%) and uninfected (22/236, 9.3%) groups were not statistically significantly different. Maternal risk factors for transmission were similar for children heterozygous or homozygous for the WT allele (CD4 cell count, 491±316 vs 526±233; positive p24 antigenemia, 12% vs 17%; cesarean delivery, 15% vs 22%; nonsignificant for all parameters).

CCR5 Genotype and Disease Progression in Children

We restricted analysis of disease progression to infected children prospectively followed up since birth (126 WT homozygotes, 26 Δ32 heterozygotes). For a mean (SD) period of 62 (36) months, the cumulative risk for the onset of stage C symptoms was significantly lower for heterozygotes than WT homozygotes (P<.004, log-rank test, Figure 1, top). The risk of development of stage C symptoms at 36 months was 9% for heterozygotes and 28% for the WT homo-
zygotes. The findings were similar for stage B symptoms (log-rank test, P<.004, Figure 1, bottom).

The proportion of children at 8 years old who had no stage B or C symptoms was 49% for the heterozygotes and 11% for the children homozygous for the WT allele (P<.003, log-rank test). Antiretroviral treatment and prophylaxis were identical in the 2 groups. The neonatal variables previously shown to have prognostic value in this cohort14-25 for the evolution of the disease in infected children were similar for children heterozygous or homozygous for the WT allele (CD4 cell count, 57% vs 55%; positive p24 antigenemia at birth, 8.3% vs 7.6%; positive culture at birth, 40% vs 31.2%; all nonsignificant).

COMMENT

The recently characterized chemokine receptor gene CCR5 and its most frequent mutation (Δ32) have become the objects of intense interest since their roles in the entry of HIV-1 into target cells were identified. This work reports the first estimation of genotype distribution of the deletion in children born to HIV-1–infected mothers. The genotypes are in Hardy-Weinberg equilibrium, suggesting the absence of deleterious genotypes. The Δ32 allele frequency in this population is quite similar to that observed in the southern European and Mediterranean populations.26 Homozygosity for a 32-bp deleted allele in the CCR5 gene protects adults from HIV-1 infection following blood or sexual exposure.1 So, it is possible that this mutated allele also has a protective effect in children born to HIV-1–seropositive mothers. Only 1 child in our cohort proved to be homozygous for the deletion, despite the large number of children tested. Therefore, this study does not allow any conclusion as to the protective effect of the deleted allele in the homozygous state. A meta-analysis based on data from international sources may be required to determine the protective effect of homozygosity on exposed newborns.

Our study does not evidence any partial resistance to HIV-1 infection among heterozygous children. The findings for heterozygous adults are discordant: Dean et al,2 Huang et al,1 and Zimmerman et al13 found no protective effect; whereas, Samson et al22 and Michael et al3 suggested the existence of a protective effect as they reported a frequency of the heterozygous state that was lower in those who were infected than in the general population or a control group. A major strength of prospective cohorts of children born to HIV-1–seropositive mothers is that infected and uninfected patients can be compared within a single group rather than using reference group comparison. Considerations of the predictive biological factors for the disease in children have mostly addressed the risk of early and severe forms, which are associated with advanced maternal disease24 and viral replication in utero.25,27 Genetic factors have previously been suggested in HIV-1–infected children, namely the HLA genotype23 and complement system genotype,25 but only in analyses of a small group. Those concerning HLA remain controversial.25

In our study, the progression of the disease was substantially reduced in CCR5Δ32 heterozygous children than in children homozygous for the WT allele. Studies of adults are not completely concordant: Dean et al13 described a clearly slower disease progression among heterozygotes, but the study was restricted to patients infected by sexual exposure. Huang et al3 and Meyer et al11 reported a similar impact on biological indicators (viral load and CD4 cell count) in a cohort of sexually infected patients but no difference between morbidity and mortality. A beneficial effect on progression was not confirmed in a study by Smith et al.16 Finally, a study by Michael et al22 suggested a lower rate of progression for heterozygous patients in whom the virus was classified as nonsymptomatic-inducing, ie, the viral strains that use the CCR5 coreceptor. Once again, the study of prospective cohorts of perinatally infected children is advantageous because it is possible to investigate putative predictive factors in homogeneous conditions, the date of infection being known for every case. Although heterozygous children were susceptible to early and severe disease, including encephalopathy, the risks of developing immune deficiency and clinical signs were lower for heterozygotes than for children homozygous for the WT allele. The low numbers of children with encephalopathy (15 of 126 WT/WT and 1 of 26 WT/Δ32 [not statistically significantly different]) in both groups did not allow any conclusion as to the putative role of the receptor in HIV-related neurologic disease. The mechanism of the protective effect is unknown21 but may be associated with a reduced expression of the receptors at the cell surface leading to decreased efficiency of HIV-1 entry and replication in CD4 T cells.23 Finally, the role of another HIV-1 receptor variant, CCR2, in the progression of the acquired immunodeficiency syndrome, still debated for adults, has to be evaluated in this situation.32,33

In conclusion, the CCR5Δ32 heterozygous genotype in children born to HIV-1–seropositive mothers does not confer resistance to infection, but it substantially slows development of HIV-related disease. The sensitivity to HIV-1 infection of children homozygous for the mutation can only be assessed by combining similar studies, as this genotype is very rare. Should this mutation be found to be protective, it would be interesting to investigate a CCR5 antagonist34 in combination with antiretroviral therapy for prevention of mother-to-child transmission.

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Figure 2.—CCR5 genotype and disease progression in 152 infected children prospectively followed up since birth. Kaplan-Meier plots for incidence of severe, class 3 immune deficiency (CD4<15%), WT/WT indicates children homozygous for the wild-type CCR5 allele (n=126); WT/Δ32, children heterozygous for wild-type CCR5 and mutated Δ32 CCR5 alleles (n=26). Log-rank test, P<.001.


