Distribution of CCR5Δ32 in Human Immunodeficiency Virus-Infected Children and Its Relationship to Disease Course

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Homozygosity for a 32-bp deletion in the CCR5 gene (CCR5Δ32) has been shown to confer resistance to infection with the macrophage-tropic strain of human immunodeficiency virus (HIV) type 1. We examined the distribution of CCR5Δ32 in 47 children (age range, 1.5 to 19 years), of whom 43 were infected with HIV, by the perinatal route (n = 41) or by the intravenous route (n = 2). The infected patients were classified as rapid progressors (RP) (n = 7) (CDC category C3 or death by 2 years of age), non-rapid progressors (NRP) (n = 17) (survival for ≥8 years after infection), or intermediate (n = 19). CCR5Δ32 heterozygosity was found in two HIV-infected children, both NRP. None of the subjects were homozygous for CCR5Δ32, and the remaining children had no evidence of CCR5Δ32. The presence of CCR5Δ32 heterozygosity in 4.8% of this, predominantly non-Caucasian population is consistent with the published distribution of the mutation. The finding that CCR5Δ32 was present only in NRP and not in any RP is in agreement with previous reports suggesting that heterozygosity for CCR5Δ32 may confer limited protection from disease progression.

Viral, immunologic, and genetic host factors are known to influence the risk of human immunodeficiency virus (HIV) infection and the rate of disease progression. Although the role of the CD4 molecule as a high-affinity receptor which is necessary but not sufficient for HIV entry has been known for a long time, it is only recently that the identity and contribution of other coreceptors in HIV infection and disease pathogenesis have begun to be recognized. One such receptor is the β-chemokine receptor 5 (CCR5) to which macrophage-tropic (M-tropic) strains of HIV-1 must bind in order to enter the CD4 cell (1, 4, 8, 9). β Chemokines RANTES, MIP-1α, MIP-1β are the natural ligands of CCR5 and inhibit the entry of M-tropic viruses into the cells (5). M-tropic non-syncytiotrobus-infecting viruses are important for establishing infection by the mucosal route and through inoculation of blood and are believed to play a role in mother-infant transmission of HIV (16). They are the predominant viruses early in the disease course. In contrast, viruses in advanced disease are predominantly T cell tropic, syncytiotrobus-infecting viruses (6, 14, 17) and utilize the other coreceptor, CXCR-4 (previously known as fusin or LESTR), which like CCR5 is a seven-transmembrane protein whose ligand has been identified as the stromal cell-derived factor (10).

A genetic mutation in the CCR5 gene consisting of 32-bp deletion (CCR5Δ32 [Δ32]) results in a nonfunctional chemokine receptor. Homozygosity for this allele (Δ32/Δ32) confers strong resistance to infection by HIV (12, 13). The Δ32 allele has also been reported to influence the rate of disease progression, although the data is conflicting (7, 11).

The frequency of the Δ32 gene in the HIV-infected pediatric population and its influence on disease progression in HIV-infected children are relatively unknown. The objectives of this study were to assess the distribution of Δ32 in a population of HIV-infected children and to study the effect of the Δ32 mutation on the course of the disease. No child was found to be homozygous for Δ32. Two infected subjects, both non-rapid progressors, were heterozygous for Δ32 (wild type [wt]/Δ32), but the Δ32 allele was not identified in any child with rapid disease progression. Although the difference in the prevalence of wt/Δ32 in rapid disease progressors compared to non-rapid disease progressors did not achieve statistical significance (P = 0.076), analysis of disease severity in this cohort suggests that Δ32 heterozygosity may contribute to slowing disease progression.

MATERIALS AND METHODS

Patients. Forty-seven pediatric patients (43 HIV infected, 2 exposed to HIV [mothers were HIV positive], and 2 not exposed to HIV [mothers were HIV negative]) were evaluated. The infected children had acquired disease through the perinatal route (n = 41) or through infected blood products (2 hemophiliacs). Children in the perinatal-infection group ranged in age from 1.7 to 18.8 years. There were 24 males and 17 females. The two hemophiliacs were 16.6 and 19.5 years of age. Ethnically there were 63% African-Americans, 15% Caucasians, 10% Latinos, 5% of mixed parentage, 5% Asians, and 2% of unknown ethnic background.

Disease severity was determined by using the CDC (Centers for Disease Control and Prevention)-defined clinical and immunologic criteria for classification of disease in children with HIV infection (3). Rapid progressors were defined as children who died or developed severe clinical disease (CDC category C3) or severe immune suppression (CDC immune category 3) by 2 years of age. Patients who survived for ≥8 years were considered non-rapid progressors; a subset of non-rapid progressors who had survived for 12 to 17 years after acquisition of perinatal infection were designated long-term survivors. Children who were neither rapid nor non-rapid progressors and ranged in age from 2 to 8 years were considered to have an intermediate disease course.

Based on disease severity, 7 (17%) perinatally infected children were classified as rapid disease progressors, and 15 (37%) were classified as non-rapid disease progressors; 7 of the non-rapid progressors were considered to be long-term survivors. There were 19 (44%) children who had an intermediate disease course. The two hemophiliacs who had survived for longer than 8 years after acquisition of infection were considered non-rapid progressors.

CCR5 genotyping. (See reference 11.) DNA was isolated from stored peripheral blood mononuclear cells of 43 HIV infected, 2 uninfected HIV-exposed, and 2 uninfected unexposed children. To detect the presence or absence of the Δ32 allele, a PCR-based assay using primers flanking the 32-bp segment was used to amplify this region of the CCR5 gene. The presence or absence of the deletion was then determined by gel electrophoresis as follows.

Brieﬂy, a portion of the CCR5 gene was ampliﬁed by PCR from genomic DNA.
and analyzed on a 4% Metaphere agarose gel (FMC BioProducts). Primers CCR5c, 5'-CAAAAGAAGGTCTCATTACACC-3', and CCR5d, 5'-CCTG TGCCCTCTCTCATTGCG-3', which flank the 32-bp deletion were used to generate wild-type and deletion fragments of 189 and 157 bp, respectively. The PCR mixture contained 0.25 mM deoxynucleoside triphosphates, 20 pmol of each primer, and 0.5 U of Taq polymerase in 1× reaction buffer (Boehringer Mannheim). Each PCR amplification consisted of 40 cycles, with the first 5 cycles consisting of 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 45 s.

HIV RNA was determined by Roche Amplicor assay.

**RESULTS**

None of the HIV-infected children had the homozygous gene for Δ32. The mutant CCR5 allele (wt/Δ32) was present in 2 (4.2%) of the 47 children—in none of the 7 rapid disease progressors; in 2 of the 17 non-rapid disease progressors (11.76%) and of the 36 children in the combined group of non-rapid progressors and those with intermediate disease course (5.5%) (Table 1). Differences in the prevalence of the mutant CCR5 gene between any of the groups were not significant by Fisher’s exact test, although there was a trend to-
some protection from development of severe HIV disease in adults (11). Interestingly, we did not find CCR5Δ32 in any of the long-term survivors in our population, indicating that the modification in the disease course achieved by a single allele is modest at best. In studies of homosexual cohorts, the frequency of wt/Δ32 in the long-term nonprogressors was found to be twice that in rapid progressors (7). In hemophiliac cohorts, on the other hand, the difference between the frequencies of the Δ32 allele in the two groups was not significant (7). This differential response may be related to differences in routes of transmission, exposure levels, or viral loads in different risk groups.

In summary, a relatively low frequency (4.8%) of the Δ32 mutation was observed in a small cohort of HIV-infected children and adolescents and was similar to the known frequency in the general nonwhite U.S. population. None of the infected children had the homozygous deletion. Children with wt/Δ32 had a relatively favorable disease course, but none of the children in our cohort who were truly long-term nonprogressors had this deletion, indicating that other host or viral factors may be important in disease pathogenesis in this population. Study of polymorphism of CCR5 and other genes in large cohorts of long-term survivors and in HIV-exposed uninfected children are warranted to determine the roles of such genetic modifications in protection from vertical infection and/or disease progression in infants with perinatal HIV exposure.

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