Prognostic Value of the Stromal Cell–Derived Factor 1 3′A Mutation in Pediatric Human Immunodeficiency Virus Type 1 Infection

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A mutation of the stromal cell–derived factor 1 gene (SDF-1 3′A) was shown to protect adults exposed to human immunodeficiency virus type 1 (HIV-1) from infection and to affect HIV disease progression in adults. The presence of this mutation in HIV-1–infected Kenyan children did not predict mother-to-child virus transmission. The SDF-1 3′A polymorphism was studied in 256 HIV-1–infected, 118 HIV-1–exposed but uninfected, and 170 unexposed and uninfected children of Italian origin, and the frequency of SDF-1 3′A heterozygosity and homozygosity in each of the 3 groups was similar. Of the 256 HIV-1–infected children, 194 were regularly followed up and were assigned to groups according to disease progression. The frequency of the SDF-1 3′A allele was substantially lower among children with long-term nonprogression than among children with rapid (P = .0329) or delayed (P = .0375) progression. We show that the presence of the SDF-1 3′A gene correlates with accelerated disease progression in HIV-1–infected children born to seropositive mothers but does not protect against mother-to-child HIV-1 transmission.

A homozygous (G-to-A) mutation at position 881 of the 3′-untranslated region of the stromal cell–derived factor 1 gene (SDF-1 3′A) has been linked to delayed progression to AIDS in adults infected with human immunodeficiency virus type 1 (HIV-1)[1]. However, several subsequent studies have shown a correlation between the presence of the SDF-1 3′A allele and accelerated progression of HIV infection to AIDS or death [2–5]. Furthermore, the relevance of the SDF-1 3′A allele to resistance to infection in adults exposed to HIV was not clear, because a significant correlation with SDF-1 3′A heterozygosity was found in only 1 of the 3 cohorts studied by Winkler et al. [1]. With regard to pediatric HIV-1 infection, one study has shown that the mother’s, but not the infant’s, SDF-1 genotype is associated with mother-to-child HIV-1 transmission [6], and another showed that the protective effect of the heterozygous form of the 32-bp deletion in the CC chemokine receptor gene (CCR5 Δ32) is restricted by SDF-1 genotype in HIV-1–infected children [7].

Patients and Methods

Patients. The present study included 544 children of Italian origin. Of these, 374 children (256 who were infected with HIV-1 and 118 who had been exposed to HIV but were uninfected) were born to HIV-1–seropositive mothers who had not received antiretroviral treatment to prevent mother-to-infant transmission because the treatment was not in use at the time of birth. None of the children was breast-fed. Children were enrolled from birth (through an Italian study on mother-to-child HIV-1 transmission) or on

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Informed consent was obtained from patients or their parents/guardians.

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being identified as infected with HIV-1 and were regularly followed up at several pediatric health care centers in northern and central Italy via the Italian Register for HIV-1 Infection in Children. One hundred seventy children who had been neither exposed to nor infected with HIV-1 were enrolled from outpatient ambulatory clinics in the same areas in northern and central Italy.

Clinical and immunologic staging of the HIV-1–infected children was defined according to the revised classification system of the Centers for Disease Control and Prevention (CDC) [8]. The 194 HIV-1–infected children included in the analysis of the relationship between the SDF-1 3’A polymorphism and disease progression were regularly followed up from birth or thereafter and were divided into groups according to the rate of disease progression, using guidelines published elsewhere [9]. Children with “rapid progression” (n = 38) included all those who developed immunodeficiency or any symptom of HIV-1 infection before 8 years of age but who had not progressed to CDC stage C3 before 2 years of age. Patients with “long-term nonprogression” (LTNP; n = 24) were those children who presented with no or mild symptoms of HIV-1 infection after 8 years of age (CDC category N1 or A1). Children with LTNP were followed up until 12–16 years of age; children with delayed progression were followed up until 3–12 years of age (mean age, 9.1 years). None of the children received highly active antiretroviral therapy or protease inhibitors during the course of the study.

**SDF-1 genotyping.** Genomic DNA was obtained by lysis of 2 x 10^5 peripheral blood mononuclear cells. The SDF-1 gene variants were obtained after polymerase chain reaction amplification of genomic DNA with reverse (AGCTTTGGTCTGGGAGTC) and forward (CAGTCACCTGGCCAGGCC) primers, followed by Msp1 digestion, as described elsewhere [1].

**Statistical analysis.** Departures from Hardy-Weinberg equilibrium in the LTNP and rapid-progression groups, and it was not significant for any comparison. Therefore, the presence of the SDF-1 3’A allele was associated with accelerated disease progression. The frequency of the genotypes was in Hardy-Weinberg equilibrium in the LTNP and rapid-progression groups, and it was not significant for any comparison.

### Results

**Effect of the SDF-1 mutation in an infant on mother-to-child HIV-1 transmission.** Our study showed that the allelic and genotypic frequencies of the SDF-1 3’A mutation among HIV-1–infected infants and HIV-1–uninfected infants who were born to seropositive mothers did not differ. Of 256 HIV-1–infected children born to seropositive mothers, 87 (34%) were heterozygous for the SDF-1 3’A allele (table 1). Similarly, 44 (37.3%) of 118 HIV-1–uninfected children born to seropositive mothers were heterozygous for the SDF-1 3’A allele. In addition, no differences were seen in the frequency of homozygosity among HIV-1–infected (19 [7.4%] of 256 children) and HIV-1–uninfected (9 [7.6%] of 118 children) children. The frequencies demonstrated among the 170 unexposed and HIV-1–negative children were similar to those seen among HIV-1–exposed children (table 1). The 3 populations analyzed were all in Hardy-Weinberg equilibrium. Therefore, the SDF-1 3’A mutation in the child, whether homozygous or heterozygous, does not seem to play a role in mother-to-child HIV-1 transmission.

**Correlation between SDF-1 3’A genotype and accelerated disease progression in children.** The relationship between the SDF-1 3’A mutation and disease progression was further studied in HIV-1–infected children (38 with rapid progression, 132 with delayed progression, and 24 with LTNP). No child in the LTNP group was homozygous for the SDF-1 3’A allele, whereas the frequency of SDF-1 3’A homozygosity was 11.4% among children with delayed progression and 7.9% among children with rapid progression (table 2); these frequencies were similar to those in the control population (mean for total study group, 8.3%) (table 1). Furthermore, SDF-1 3’A heterozygosity was also underrepresented in the LTNP group, compared with the other 2 groups of children (25% in children with LTNP vs. 30.3% and 42.1% in children with delayed and children with rapid progression, respectively). The frequency of the SDF-1 3’A allele was, therefore, significantly lower in the LTNP group than it was in the delayed-progression (P = .0375) and rapid-progression (P = .0329) groups. As a consequence, the frequency of the wild-type SDF-1 genotype was higher among children with LTNP than among children with rapid progression (P = .05). Therefore, the presence of the SDF-1 3’A mutation was associated with accelerated disease progression.

The frequency of the genotypes was in Hardy-Weinberg equilibrium in the LTNP and rapid-progression groups, and it was not significant for any comparison.

### Table 1. Stromal cell–derived factor 1 (SDF-1) allele and genotype frequencies among human immunodeficiency virus type 1 (HIV-1)–infected and HIV-1–uninfected Italian children.

<table>
<thead>
<tr>
<th>Subject group</th>
<th>No. of subjects</th>
<th>No. (%) of subjects with given SDF-1 genotype</th>
<th>No. (%) of given SDF-1 alleles in the tested population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>wt/wt</td>
<td>3’A/wt</td>
</tr>
<tr>
<td>HIV-1 infected</td>
<td>256</td>
<td>150 (58.6)</td>
<td>87 (34)</td>
</tr>
<tr>
<td>HIV-1 exposed, uninfected</td>
<td>118</td>
<td>65 (55.1)</td>
<td>44 (37.3)</td>
</tr>
<tr>
<td>HIV-1 unexposed, uninfected</td>
<td>170</td>
<td>94 (55.3)</td>
<td>59 (34.7)</td>
</tr>
<tr>
<td>Total</td>
<td>544</td>
<td>309 (56.8)</td>
<td>190 (34.9)</td>
</tr>
</tbody>
</table>

**NOTE.** wt, wild type; wt/wt, homozygous wild-type gene; 3’A/wt, heterozygous for the 3’A mutation of the SDF-1 gene; 3’A/3’A, homozygous for the 3’A mutation of the SDF-1 gene. P was not significant for any comparison.
significantly different from the expected values in the delayed-progression group ($P > .05$). This was ascribed principally to the high frequency of the homozygous mutated genotype in this group.

**Combined analysis of SDF-1 3'A and CCR5 Δ32 gene mutations.** The results for the SDF-1 3'A gene mutation were analyzed in combination with previously published results for the CCR5 Δ32 deletion in the same cohort of children [10]. As shown in table 3, the children were divided into 4 phenotype groups: 3'A+/Δ32`-, 3'A-Δ32`, 3'A+/Δ32`, and 3'A-Δ32`. None of the children with rapid progression carried a CCR5 Δ32 phenotype (table 3), confirming that this mutation has a role in delaying disease progression. The presence of the SDF-1 3'A mutation in the absence of the CCR5 Δ32 deletion (3'A+/Δ32`) showed a distribution among disease progression groups similar to that of the SDF-1 3'A(3'A`) phenotype. Furthermore, the absence of the SDF-1 3'A mutation and the presence of the CCR5 Δ32 mutation (3'A-Δ32`) strongly correlated with LTNP (statistical analysis indicated in footnote to table 3). The presence of both mutations (3'A+/Δ32`) was seen only in 5 children with delayed progression.

#### Table 2. Stromal cell–derived factor 1 (SDF-1) allele and genotype frequencies, according to disease progression, in 194 Italian children infected with human immunodeficiency virus type 1.

<table>
<thead>
<tr>
<th>Disease progression</th>
<th>No. of subjects</th>
<th>No. (%) of subjects with given SDF-1 genotype</th>
<th>No. (%) of given SDF-1 alleles in the tested population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>wt/wt</td>
<td>3'A/wt</td>
</tr>
<tr>
<td>Rapid</td>
<td>38</td>
<td>19 (50)</td>
<td>16 (42.1)</td>
</tr>
<tr>
<td>Delayed</td>
<td>132</td>
<td>77 (58.3)</td>
<td>40 (30.3)</td>
</tr>
<tr>
<td>Long-term nonprogression</td>
<td>24</td>
<td>18 (75)</td>
<td>6 (25)</td>
</tr>
</tbody>
</table>

NOTE. wr, wild type; wt/wt, homozygous wild-type gene; 3'A/wt, heterozygous for the 3'A mutation of SDF-1 gene; 3'A/3'A, homozygous for the 3'A mutation of the SDF-1 gene.

#### Discussion

We show that heterozygosity for the SDF-1 3'A mutation in infants does not correlate with protection from mother-to-child transmission of HIV-1 in an Italian population, a finding that is in agreement with a recently published report on a Kenyan population [6]. In addition, the presence of children who were homozygous for SDF-1 3'A in our cohort allowed us to rule out the possibility that this genotype plays a role in mother-to-child HIV-1 transmission, which could not be addressed in the Kenyan study [6] because the genotype was not found in that cohort.

The overall frequency (8.3%) of the homozygous SDF-1 3'A condition for our cohort is in accordance with that estimated for the Caucasian population and with that of the population of northern and central Italy in previous studies of adults [1, 5]; however, the frequency is substantially higher than that described for the Italian population of Sardinia (0%) [11]. This is not surprising, because a great genetic distance between Sardinian and continental Italian individuals has been reported for several other polymorphisms [12].

#### Table 3. Combined effect of the 32-bp deletion in the CC chemokine receptor 5 gene (CCR5 Δ32) and stromal cell–derived factor 1 (SDF-1) wild-type phenotypes on protection against rapid progression to AIDS in Italian children infected with human immunodeficiency virus type 1.

<table>
<thead>
<tr>
<th>Disease progression</th>
<th>No. of subjects</th>
<th>3'A</th>
<th>Δ32`</th>
<th>3'AΔ32`</th>
<th>3'A-Δ32`</th>
<th>3'AΔ32`</th>
<th>3'A-Δ32`</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid</td>
<td>38</td>
<td>19 (50)</td>
<td>0</td>
<td>19 (50)</td>
<td>0</td>
<td>0</td>
<td>19 (50)</td>
</tr>
<tr>
<td>Delayed</td>
<td>132</td>
<td>55 (41.7)</td>
<td>11 (8.3)</td>
<td>50 (37.9)</td>
<td>6 (4.5)</td>
<td>5 (3.8)</td>
<td>71 (53.8)</td>
</tr>
<tr>
<td>Long-term nonprogression</td>
<td>24</td>
<td>6 (25)`</td>
<td>6 (25)`</td>
<td>6 (25)`</td>
<td>6 (25)`</td>
<td>0</td>
<td>12 (50)</td>
</tr>
</tbody>
</table>

NOTE. 3'A, presence of the SDF-1 3'A mutation; 3'A, absence of the SDF-1 3'A mutation; Δ32`, presence of the CCR5 Δ32 mutation; Δ32`, absence of the CCR5 Δ32 mutation.

- Long-term nonprogression vs. rapid progression, $P = .0506$; long-term nonprogression vs. delayed progression, $P = .12$.
- Long-term nonprogression vs. rapid progression, $P = .0012$; long-term nonprogression vs. delayed progression, $P = .0159$.
- Long-term nonprogression vs. rapid progression, $P = .0506$; long-term nonprogression vs. delayed progression, $P = .0005$.
- $P$ was not significant for any comparison.
Overwhelming evidence indicates that a large proportion of neonates are infected with MT-2–negative viruses (i.e., viruses capable of using the CCR5 receptor only) [13, 14]. Therefore, it is not surprising that SDF-1, the ligand of CXC chemokine receptor 4 (CXCR4), may not affect mother-to-child transmission of R5 viruses. However, it is possible that SDF-1 has an inhibitory effect on the transmission of X4 viruses harbored by the mother. Indeed, the role of the G-to-A mutation in the 3'-untranslated region of SDF-1 on the protein function is still unclear, and, thus, the possible role of this mutation in HIV-1 infection is also unclear. Study of the SDF-1 3'A mutation in children born to mothers harboring an X4 virus would shed light on the mutation's role in vivo.

We show that the presence of the SDF-1 3'A gene correlates with rapid disease progression in HIV-1–infected children born to seropositive mothers. This finding is in agreement with most studies of the mutation in HIV-infected adults, which have shown an association between the SDF-1 3'A homozygous mutation and accelerated onset of AIDS or progression to death after AIDS diagnosis [2–5]. All of these studies are in disagreement with the first study of this subject, by Winkler et al. [1]. Furthermore, the recent study by Sei et al. [7] did not show any significant difference in the frequency of AIDS development in children during the first 3 years of life in relation to SDF-1 3'A genotype. This group of children corresponds to the children in our study who had rapid progression (i.e., children who developed AIDS and severe immunodeficiency by 2 years of age). However, the overall frequency of the SDF-1 3'A mutation is higher among the Italian children in our study than among the 58 Caucasian children in the United States studied by Sei et al. [7] (41.4% vs. 34.5%, respectively). Indeed, population-based frequencies may account for this difference and must be investigated in larger studies.

The similarity between distribution of the SDF-1 3'A genotype and distribution of the SDF-1 3'A genotype in the absence of the CCR5 Δ32 mutation (3'A+/Δ32−) indicates that the CCR5 Δ32 phenotype does not affect the presence of the SDF-1 3'A mutation. However, the CCR5 Δ32 phenotype appears to be restricted by the SDF-1 3'A mutation, because both mutations were rarely present in the same subject. These data are in line with the data reported by Sei et al. [7]; however, the small number of children analyzed in both studies may have affected the analyses.

HIV-1 disease progression has been associated with a switch of the viral phenotype from non-syncytium inducing to syncytium inducing. Marechal et al. [15] described multiple and opposite effects of SDF-1, including inhibition of the entry of X4 viruses and stimulation of proviral gene expression of R5 viruses in vitro. Therefore, it is tempting to speculate that a change in viral phenotype may be favored by the presence of the mutated form of the SDF-1 gene, which would lead to the appearance of CXCR4-using viruses. However, none of the studies that investigated this found a correlation between the presence of the mutated gene and the viral phenotype [4, 5]. We have characterized the biological phenotype, defined as the tropism of the virus isolate for MT-2 cells, of a series of virus isolates from 32 children with delayed progression and 10 children with LTNP from the same cohort that was observed in the present study [10]. Only 1 of the 10 children with LTNP developed an MT-2–tropic virus isolate; this child carried the wild-type SDF-1 gene (data not shown). However, 15 (46.9%) of the 32 children with delayed progression developed a syncytium-inducing virus. Of this group, 6 children were wild-type homozygotes, 7 were heterozygotes, and 2 were homozygous for the SDF-1 3'A mutation. Although the frequency of MT-2–tropic virus isolates was lowest in the group of children who were homozygous for the SDF-1 3'A mutation (6.2%), we did not observe any statistically significant difference between the frequencies of the 3 genetic conditions and the frequency with which a syncytium-inducing virus was isolated. Notably, 3 of the children with delayed progression and 4 of the children with LTNP were also heterozygous CCR5 Δ32 carriers. Because this cohort is relatively small, larger studies should be considered to definitively assess the relevance of the combined genetic polymorphism in virus phenotype variation.

In conclusion, our data strongly suggest that the SDF-1 3'A gene does not protect perinatally exposed children from HIV-1 infection but does play a role in accelerating disease progression in infected children. The rapidity and simplicity of the assay for the detection of the mutation favors the use of this parameter as a marker of disease progression. It is feasible to think that the association between multiple parameters, such as virus load, CD4 cell count, and host genetic markers, may (as with the CCR5 gene, the SDF-1 gene, and possibly others) substantially increase the possibility of predicting disease outcome in children.

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References


