CONCISE COMMUNCIATION

# Shorter Survival of *SDF1-3′A/3′A* Homozygotes Linked to CD4+ T Cell Decrease in Advanced Human Immunodeficiency Virus Type 1 Infection

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**The *SDF-1* 3′A allelic polymorphism has been reported to influence either positively or negatively the progression of human immunodeficiency virus type 1 (HIV-1) disease. Therefore, the *SDF-1* genotype of 729 HIV-1–infected individuals pooled from 3 distinct cohorts was determined. A statistically nonsignificant association between the *SDF1-3′A/3′A* genotype and accelerated disease progression was evident among seroconverters ($n = 319$), but a striking correlation of decreased survival after either diagnosis of AIDS according to the 1993 definition or loss of CD4+ T cell counts <200 was observed. The relative hazards for *SDF1-3′A/3′A* homozygotes, compared with heterozygotes and wild-type homozygotes were 2.16 ($P = .0047$), for time from diagnosis according to the 1993 Centers for Disease Control and Prevention AIDS case definition (AIDS-'93) to death, and 3.43 ($P = .0001$), for time from CD4+ T cells <200 to death. Because no difference in survival was observed after diagnosis according to AIDS-'87, the association of the *SDF1-3′A/3′A* genotype with the accelerated progression of late-stage HIV-1 disease appears to be explained for the most part by the loss of CD4+ T lymphocytes.**

An increasing number of genetic polymorphisms of chemokine and chemokine-receptor genes have been related to both the risk of human immunodeficiency virus type 1 (HIV-1) transmission and disease progression (for review, see [1]). A polymorphism in conserved sequences of the 3′ untranslated region of the gene encoding the CXC chemokine SDF1-$\beta$ (*SDF1-3′A*), the unique ligand of the HIV-1 coreceptor CXCR4, was reported to delay the onset of AIDS when present in the homozygous configuration, particularly in late-stage HIV-1 disease [2]. The authors proposed that this polymorphism could result in the increased production of SDF-1, thereby preventing the appearance of more aggressive X4 HIV-1 [2]. These observations were not confirmed, however, in a cohort of 1090 subjects from the US Tri-Service HIV Natural History Study [3] and in 344 cases from the Amsterdam cohort [4]. In the Tri-Service cohort, *SDF1-3′A/3′A* subjects showed a significantly accelerated disease progression toward death and a weakly significant worsening of progression to AIDS, using the 1987 Centers for Disease Control and Prevention (CDC) AIDS case definition (AIDS-'87) [5]. These conflicting observations may depend, at least in part, on the low frequency of the *SDF1-3′A/3′A* genotype among whites (4%–5%) [2].

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In the present study, we investigated the association of *SDF1* genotype on disease progression and survival in 729 seropositive patients enrolled in 3 distinct cohorts.

## Methods

*Study cohorts.* The San Francisco Men's Health Study is a population-based cohort of 1,034 single, mostly white men enrolled in San Francisco showing the highest AIDS incidence in 1984 and followed every 6 months until 1995. Genotyping was available for 424 individuals, of whom 377 were seroprevalents. In addition, 249 infected individuals from the Swiss HIV Cohort (SHC) and 56 long-term nonprogressors (LTNPs) of the Milan cohort were included in the study.

The individuals were followed for a median of 8.23, 7.61, and 12 years in the SFMHS, SHC, and Milan cohorts, respectively, and their median ages were 34, 25, and 23.5 years. There were 1, 83, and 26 intravenous drug users; 416, 95, and 15 male homosexuals; 0, 69, and 3 heterosexuals, and 0, 2, and 12 hemophiliacs in the SFMHS, the SHC, and the Milan cohorts, respectively. There were 661 whites, 22 Hispanics, and 26 blacks.

The following end points were considered for the analysis: (1) $CD4^+$ T cell counts <200 cells/$\mu$L; (2) the AIDS-'87 case definition [5]; (3) the 1993 CDC AIDS case definition (AIDS-'93), adding the loss of $CD4^+$ T lymphocytes <200 cells/$\mu$L to the AIDS defining conditions of AIDS-'87; and (4) death.

Plasma HIV-1 RNA concentrations were available from 674 subjects [6, 7]. Viral isolation and phenotyping were performed as described elsewhere [8, 9].

CCR5 *and* CCR2 *genotyping.* The *CCR2* and *CCR5* genotypes were determined as described elsewhere for the SFMHS cohort [6, 8] and for the SHC and Milan cohorts [7, 10].

SDF1 *genotyping.* Subjects from the SFMHS cohort were genotyped by polymerase chain reaction (PCR)—restriction fragment length polymorphism (RFLP), as reported elsewhere [2]. For the SHC and Milan cohorts, an allelic discrimination assay based on the allele-specific degradation of 2 internally quenched fluorescent probes during the PCR reaction was used [11]. Wild-type–specific probe sequence was 5′-(FAM) AGG CAG ACC CGG CTC CCA TG (TAMRA)-3′, where FAM is 6-carboxyfluorescein, and TAMRA is 6-carboxytetramethylrhodamine; the *SDF1-3′A*–specific probe sequence was 5′-(TET) AAG AGG CAG ACC TGG CTC CCA TGT (TAMRA)-3′, where TET is tetrachloro-6-carboxy-fluorescein. The primer sequences were (sense) 5′-CCA GTC AAC CTG GGC AAA G-3′ and (antisense) 5′-TGC CTC AGC TCA GGG TAG C-3′. The PCR reaction was performed according to the manufacturer's instructions on an ABI PRISM 7700 Sequence Detector System (PE Biosystems, Foster City, CA) and the dedicated TaqMan PCR Core Reagent Kit (PE Biosystems, Foster City, CA); the 1/1 specific probe (200 nM), the 30 A–specific probe (100 nM), the 2 primers (0.25 mM each), and 100–200 ng of DNA were extracted from peripheral blood mononuclear cells. Some test samples, plus all samples without a clear-cut signal level in at least 1 of the 2 fluorescent signals, were also characterized by the published PCR-RFLP method [2] as control; all of the 80 doubly tested genotype assignments were concordant.

*Statistical analysis.* Departures from Hardy-Weinberg equilibrium and differences in categorical variables were assessed by $\chi^2$ test or Fisher's exact test when appropriate. Plasma HIV-1 RNA levels were log transformed before analysis and compared with SDF1 genotype by covariance analysis. The time elapsed before reaching different immunological and clinical end points was analyzed by use of Kaplan-Meier survival curves and the Cox proportional-hazard regression models.

## Results

SDF1 *genotype distribution.* Twenty-two (5%) SFMHS participants were homozygous for *SDF1-3′A/3′A,* whereas 135 (32%) were heterozygous, and 267 (63%) were *SDF1* +/+. In the SHC, *SDF1-3′A* homozygosity was found in 12 (4.8%) subjects and heterozygosity in 76 (30.5%) subjects, whereas 161 subjects were *SDF1* +/+; in the Milan cohort, the frequencies were 3 (5%), 26 (46%), and 27 (48%) for *3′A/3′A,* +/*3′A,* and +/+, respectively. Each cohort and the grouped study population were in Hardy-Weinberg equilibrium (*P* values ranging from .304 to .439; $\chi^2$ test). Genotype distribution and the effects of other genetic polymorphisms involved in HIV disease progression (*CCR5-Δ32* and *CCR2-64I*) of these cohorts have been published elsewhere [6–8, 10].

SDF1 *genotype and progression to AIDS.* All the statistics were stratified by cohort, to correct for potential hidden confounding factors. Kaplan-Meier survival curves of the combined cohorts with the results of univariate Cox proportional hazards analyses are shown in table 1. Because the same pattern of progression was consistently observed in both *SDF1-*+/+ and *SDF1-*+/*3′A* individuals, these two groups were analyzed together. A modest increase in the relative hazard (RH) for *SDF1-3′A/3′A* individuals was observed when AIDS-'87 and death were considered as end points, but the differences were not significant. When the analyses were repeated exclusively on the seroconverter subset (table 1), as suggested elsewhere [12], the RH of the homozygous genotype on the various end points was stronger, although still not significant (figure 1).

All the analyses were repeated after adjustment for *CCR5* and *CCR2* genotypes, age, risk factor, race, therapy, and seroincidence status (i.e., seroprevalents vs. seroconverters). After these adjustments, the overall association of *SDF1-3′A/3′A* on disease progression did not change (table 1), although a further increase of the RH of *SDF1-3′A/3′A* seroconverters was noted.

SDF1 *genotype and survival after AIDS diagnosis.* We next tested whether the *SDF1* genotype could influence the late stages of infection, by analyzing survival after diagnosis of AIDS according to either AIDS-'87, AIDS-'93, or loss of circulating $CD4^+$ T lymphocytes <200 cells/$\mu$L. A gradation of survival reduction was observed for time to death after AIDS-'87, AIDS-'93, and $CD4^+$ T cell counts <200, with RH values of 1.19 (*P* = .522), 2.21 (*P* = .0034), and 3.18 (*P* = .0003), respectively (table 1 and figure 1). Thus, RH values increased and gained significance shifting from AIDS-'87 to $CD4^+$ T cell

**Table 1.** Effects of the *SDF1-3′A/3′A* genotype on progression to AIDS and from AIDS to death in Cox proportional hazard analyses.

| | *SDF1-3′A/3′A* vs. *3′A/+* and *+/+* | | | | | *SDF1-3′A/3′A* vs. *3′A/+* and *+/+*[a] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| End point | *n* | Events | RH | CI | *P* | *n* | Events | RH | CI | *P* |
| CD4<200 | | | | | | | | | | |
| All | 692 | 314 | 0.84 | 0.48–1.47 | .539 | 681 | 310 | 0.761 | 0.44–1.33 | .339 |
| Seroconverters | 315 | 99 | 1.46 | 0.58–3.69 | .418 | 314 | 99 | 1.65 | 0.66–4.14 | .282 |
| AIDS-'93 | | | | | | | | | | |
| All | 699 | 380 | 1.08 | 0.68–1.72 | .744 | 688 | 375 | 1.01 | 0.63–1.60 | .980 |
| Seroconverters | 318 | 114 | 1.56 | 0.67–3.61 | .299 | 317 | 114 | 1.81 | 0.78–4.18 | .166 |
| AIDS-'87 | | | | | | | | | | |
| All | 705 | 311 | 1.27 | 0.78–2.07 | .337 | 693 | 306 | 1.21 | 0.74–1.98 | .450 |
| Seroconverters | 319 | 71 | 1.87 | 0.67–5.19 | .232 | 318 | 71 | 2.23 | 0.79–6.30 | .131 |
| Death | | | | | | | | | | |
| All | 711 | 273 | 1.26 | 0.75–2.12 | .388 | 699 | 268 | 1.18 | 0.70–1.99 | .543 |
| Seroconverters | 319 | 49 | 2.06 | 0.63–6.76 | .234 | 318 | 49 | 2.38 | 0.70–8.04 | .163 |
| Time to death since | | | | | | | | | | |
| AIDS-'87 | 309 | 261 | 1.19 | 0.70–2.00 | .522 | 304 | 256 | 1.13 | 0.67–1.91 | .648 |
| AIDS-'93 | 374 | 259 | 2.21 | 1.30–3.75 | .0034 | 369 | 255 | 2.16 | 1.27–3.68 | .0047 |
| CD4 <200 | 314 | 211 | 3.18 | 1.71–5.92 | .0003 | 310 | 208 | 3.43 | 1.83–6.45 | .0001 |

NOTE. AIDS-'87, 1987 Centers for Disease Control and Prevention (CDC) AIDS case definition; AIDS-'93, 1993 CDC AIDS case definition; CI, 95% confidence interval; RH, relative hazard.

[a] Adjusted for *CCR5* and *CCR2* genotypes, age, risk factor, race (categorized as whites vs. other races), therapy, and seroincidence status (only for the first 4 end points and for the entire data set).

counts <200. Adjusting the analysis for the same confounding factors above described did not alter the results (table 1).

SDF1 *genotype and viral phenotype.* Because SDF-1 binds to CXCR4, we tested whether the *SDF1-3′A* genotype could influence viral evolution from nonsyncytium-inducing (NSI) to syncytium-inducing (SI) strains (i.e., from CCR5 to CXCR4 usage). Ninety-three NSI viruses versus 49 SI were isolated in a sample of 142 *SDF1-+/+* patients. Among 70 *SDF1-+/3′A* patients, 48 had NSI and 22 had SI viruses, whereas among 15 *SDF1-3′A/3′A* patients, 11 had NSI and 4 had SI viruses (data not shown). The differences among these frequencies are not significant ($P = .780$, $\chi^2$ test), which suggests that the *SDF1* genotype did not influence the phenotype of HIV-1 primary isolates.

SDF1 *genotype and viremia.* A single plasma HIV-1 RNA level determination was available for each subject; the median viral loads were 30,200, 23,174, and 5688 copies of HIV-1 RNA/mL for the SFMHS, the SHC, and the Milan cohorts, respectively. We compared the mean log transformed values of viremia adjusting for cohort, because they differed in terms of time from enrollment of the titered sample. Like viral phenotype, this parameter was not influenced by the *SDF1* genotype ($P = .223$, covariance analysis; data not shown).

## Discussion

In the present study, we have demonstrated that *SDF1-3′A/3′A* homozygosity weakly accelerates HIV-1 disease progression, a result consistently observed by all the clinical end points considered. More important, this genotype was linked to a shorter survival from the time of AIDS diagnosis to death if $CD4^+$ T cell counts <200 were included among the diagnostic criteria.

The effect of the *SDF1* genotype on survival of HIV-1–infected subjects is highly controversial, ranging from delay to acceleration of the disease progression [2–4]. Because these discrepancies are likely influenced by the low frequency of *SDF1-3′A/3′A* whites, the analysis of multiple cohorts, as here reported, should help clarify this issue. The *SDF1* genotype was tested against various markers of disease progression. We found a trend toward faster progression for *SDF1-3′A/3′A* individuals, compared with *SDF1+/3′A* and *+/+* subjects. This trend was more pronounced when only seroconverters were considered. In this regard, the confounding effect of seroprevalents in studies on the role of *CCR5* and *CCR2* genotypes on disease progression has been discussed elsewhere [12]. In contrast to Winkler et al. [2], but consonant with the results reported here, two reports [3, 4] have elsewhere described an association of the *SDF1-3′A* homozygous genotype with accelerated disease progression. Of note, when analyses were performed on a cohort whose design mitigated confounding variables [3], the association with accelerated progression was clearly visible. The recently reported role of race-specific *CCR5* haplotypes on HIV-1 pathogenesis further underscores the importance of controlling for confounding factors [13].

We observed a strong association between the *SDF1-3′A/3′A* genotype and survival reduction after either AIDS-'93 or $CD4^+$ T cell counts <200. In contrast, all the SDF1 genotypes were indistinguishable with respect to survival after AIDS-'87. We interpret this association as a linkage between the *SDF1-3′A/3′A* genotype and the progressive immunodeficiency occurring after erosion of $CD4^+$ T lymphocytes <200 cells/$\mu$L, resulting in increasing incidence of opportunistic infections and tumors.

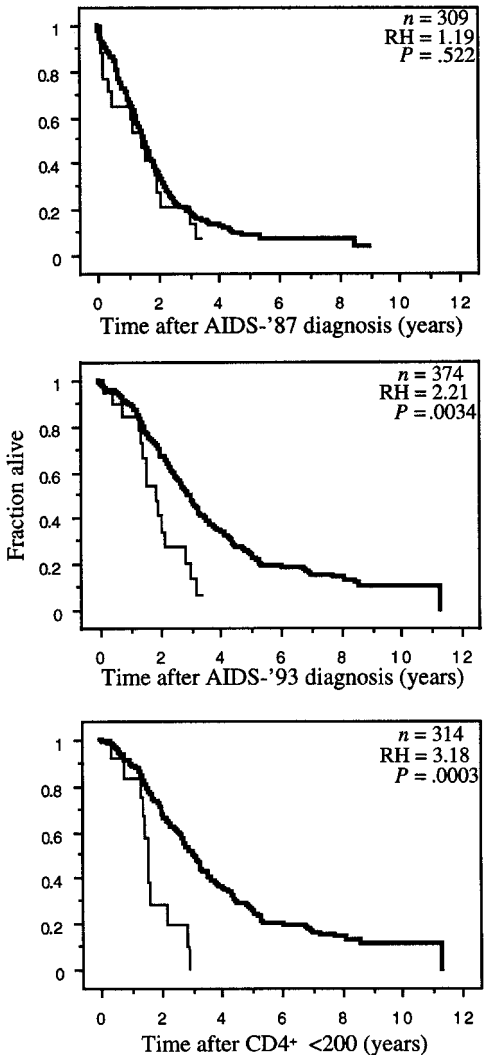


**Figure 1.** *SDF1-3′A/3′A* genotype and time from AIDS to death. The thick line represents *SDF1-+/+* plus *SDF1-+/3′A* subjects, and the thin line represents *SDF1-3′A/3′A* subjects. *P* values were based on the Cox proportional-hazards model (univariate test), with data stratified by cohort. Summary statistics are shown in table 1. AIDS-'87, 1987 Centers for Disease Control and Prevention (CDC) AIDS case definition; AIDS-'93, 1993 CDC AIDS case definition; RH, relative hazard.

In support of this interpretation, HIV disease progression in seroconverters with peripheral $CD4^+$ T cell counts >200 cells/$\mu$L was not significantly accelerated (RH = 1.65; $P = .282$). When a multivariate analysis with stepwise selections of the variables was performed, only the *SDF1-3′A/3′A* genotype was identified as a prognosticator of disease progression after AIDS-'93 or $CD4^+$ T cell counts <200 ($P = .0003$ and $P = .0006$, respectively), whereas other strong predictors of progression, such as plasma HIV RNA and viral phenotype, were forced out of the model.

Unlike *CCR5-Δ32,* the biological correlates of the *SDF1-3′A/3′A* genotypes are currently unknown. Since SDF-1 is the unique ligand of CXCR4, it can be speculated that an altered expression consequent to the recessive genotype could influence the evolution of HIV quasi species in vivo by changing the rate of NSI/SI viral variants. In this regard, we and others [14] have observed that *CCR2-64I,* both in heterozygotic and homozygotic configuration, was positively correlated to a predominance of SI over NSI variants in 2 (SFMHC and Milan cohorts) of the 3 cohorts here studied (authors' unpublished data). In contrast, no correlation was observed between *SDF1* genotypes and prevalence of SI/NSI primary isolates, which confirms previous reports [10, 15]. Because immunological parameters, including $CD4^+$ T cell counts and concentrations of soluble cytokine receptors and neopterin, are reported to be stronger predictors of disease progression than viremia in advanced infection [16], we believe that the influence of the *SDF1-3′A* allelic variant on disease progression is more likely related to the immune mechanisms responsible for, or affected by, $CD4^+$ T cell depletion than to viral replication per se.

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