

No Evidence for an Effect of the CCR5 $\Delta 32/+$ and CCR2b 64I/+ Mutations on Human Immunodeficiency Virus (HIV)-1 Disease Progression among HIV-1–Infected Injecting Drug Users

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The relationship between CCR5 and CCR2b genotypes and human immunodeficiency virus (HIV)-1 disease progression was studied among the 108 seroconverters of the Amsterdam cohort of injecting drug users (IDUs). In contrast to earlier studies among homosexual men, no effect on disease progression of the CCR5 $\Delta 32/+$ and the CCR2b 64I/+ genotypes was found, when progression to AIDS, death, or a CD4 cell count $<200/\mu\text{L}$ was compared by a Cox proportional hazards model. Furthermore, CD4 cell decline (by a regression model for repeated measurements) and virus load in the first 3 years after seroconversion did not differ between the CCR5 and CCR2b wild type and heterozygous genotypes. A nested matched case-control study also revealed no significant effect of the CCR5 and CCR2b mutations. Immunologic differences between IDUs and homosexual men may account for the observed lack of effect. Alternatively, difference in transmission route or characteristics of the HIV-1 variants that circulate in IDUs could also explain this phenomenon.

Several coreceptors other than CD4, belonging to different subgroups of a seven-transmembrane G protein-coupled chemokine receptor, have been identified that are necessary for human immunodeficiency virus type 1 (HIV-1) to enter cells. The α -chemokine receptor CXCR4 serves as a coreceptor for T cell line-tropic or syncytium-inducing (SI) isolates [1], whereas the β -chemokine receptor CCR5 appears to be the major coreceptor for entry of macrophage-tropic or non-SI (NSI) variants of the virus [2–4]. The β -chemokine receptors CCR2b and CCR3 are also used by HIV-1 to enter CD4 cells, although to a much lesser extent [5].

Different mutations in the genes encoding for the CCR5 and CCR2b receptors have been shown to exist. A 32-bp deletion

($\Delta 32$) in the gene encoding for the CCR5 receptor, resulting in reduced CCR5 cell surface expression [6], can be found among $\sim 15\%$ of whites but is found with a much lower frequency in other ethnic groups [7]. Subjects homozygous for the deletion, occurring in $\sim 1\%$ of whites, are protected against HIV-1 infection [8], although this protection is not absolute. Several studies published thus far have found that subjects heterozygous for the $\Delta 32$ mutation have a delayed HIV-1 disease progression [7, 9, 10].

Recently, a single point mutation (T \rightarrow A) at position 303 in the gene encoding for CCR5 has been shown to be associated with resistance to HIV-1 [11]. The effect of this mutation on HIV-1 disease progression remains to be clarified.

A mutation has also been described for the CCR2b receptor gene occurring with an allele frequency of ~ 0.098 in whites and 0.151 in Africans [12]. The mutation leads to a substitution of isoleucine instead of valine at position 64 in the first transmembrane region. Results of a few studies on the role of the CCR2b genotype in disease progression also show a favorable role of the CCR2b 64I/+ genotype in HIV-1 disease progression [12–15]. The biologic mechanism for this phenomenon is not clear, since primary HIV-1 variants do not use CCR2b as a coreceptor, and the mutation is located in a transmembrane region. It has been suggested that the CCR2b 64I mutation directly or indirectly influences the CCR5 pathway. One interesting recent observation in this respect is that the mutant CCR2b allele is in complete linkage disequilibrium with another point mutation in the CCR5-noncoding region [14].

The association between CCR5 and CCR2b genotypes and HIV-1 disease progression has thus far mainly been studied in

Received 18 May 1998; revised 17 November 1998.

Informed consent was obtained from all participants. The study was approved by, and conducted according to the guidelines of, the Medical Ethical Committee of the Municipal Health Service.

This study was performed as part of the Amsterdam Cohort Studies on AIDS, a collaboration between the Municipal Health Service, the Academic Medical Centre, and the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam.

Financial support: Netherlands Foundation for Preventive Medicine as part of the Stimulation Program on AIDS Research of the Program Committee for AIDS Research (grant 28-1258).

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The Journal of Infectious Diseases 1999;179:825–31

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0022-1899/99/7904-0009\$02.00

cohorts of homosexual men or hemophiliacs and not separately in cohorts of injecting drug users (IDUs). However, reported differences in natural history of HIV-1 infection among different exposure groups—for example, the fact that switching to SI HIV-1 variants, which use CXCR4 as a coreceptor, occurs less frequently among IDUs [16]—make investigating the role of CCR5 and CCR2b genotypes in HIV-1 disease progression in HIV-1-infected IDUs worthwhile. Therefore, our present study focuses on the role of the CCR5 $\Delta 32$ and the CCR2b 64I mutations in HIV-1 disease progression among the participants of the Amsterdam cohort of IDUs.

Methods

Subjects. Data were collected from the Amsterdam cohort study on HIV and AIDS among drug users; this study began in 1985 [17]. Participants are asked to return every 4 months for an interview, clinical examination, and blood sampling. Data are gathered regarding drug use, sexual behavior, medical conditions, HIV treatment, immunologic status, and HIV-1 status. Information on AIDS diagnosis and date or cause of death is provided by hospital-based physicians, by the national AIDS register, or by registers of death.

During follow-up of the cohort study, a total of 81 participants seroconverted for HIV antibodies (i.e., prospective seroconverters). The median time between the last negative test and the first positive test for this group of prospective seroconverters was 3.9 months (interquartile range, 3.7–5.1). Thirty-seven subjects entered the cohort HIV-1-positive, with a negative test result before entry (i.e., retrospective seroconverters). Negative test results were obtained from samples taken for hepatitis B virus serology and were unrelated to HIV-1 testing. The median interval between the last negative and first positive tests was 4.1 years (interquartile range, 1.8–5.4) for these seroconverters. Four subjects reported having never injected and were excluded. From 6 participants, no samples for chemokine receptor genotyping were available. Therefore, in the present study, the population consisted of 108 IDUs.

To further explore the relationship between CCR5 and CCR2b chemokine receptor genotypes and HIV-1 disease progression, a nested case-control study was done among HIV-positive participants of this cohort study. Cases were long-term survivors who remained AIDS-free for >9 years, with CD4 cell counts >400/ μ L after >8 years of follow-up. Controls were individually matched progressors who developed AIDS within 2–7 years. Subjects were matched by age (± 10 years), sex, and HIV-1 serostatus at entry in the cohort study and CD4 cell count (± 250 cells/ μ L) in the second year after seroconversion or, if not available, the year of the first HIV-1-positive cohort visit. Nine long-term survivors were identified who were matched with 2 controls (although for 2 long-term survivors only 1 matched progressor could be found).

Receptor genotyping. Genomic DNA was isolated from cryopreserved peripheral blood mononuclear cells (PBMC) (Qiagen blood kit; Qiagen, Chatsworth, CA) and was analyzed by use of polymerase chain reaction (PCR) with primers. Details on CCR5 genotyping have been published elsewhere [18]. For CCR2b, the primers CCR2-1A (sense, position 170–187 in CCR2b, 5'-TTGTGGGCAACATGATGG-3') and CCR2-1Z (antisense, po-

sition 279–298 in CCR2b, 5'-GAGCCACAATGGGAGAGTA-3') were used [12]). Samples were amplified with 1 U of Taq polymerase (Promega, Madison, WI) in the provided buffer with a final MgCl₂ concentration of 3 mmol/L. PCR conditions for CCR2b genotyping comprised 5 min of denaturation at 95°C; 35 cycles of 30 s at 95°C, 45 s at 48°C (slope 2°C/s), and 30 s at 72°C; and 5 min of elongation at 72°C in a thermocycler (Uno II; Biometra, Göttingen, Germany). Products of PCR were subjected to restriction analysis with *Bsa*BI for 2 h at 60°C (New England Biolabs, Beverly, MA) and analyzed on a 4% Nusieve gel (FMC Bio-Products, Rockland, ME).

Immunologic and virologic assays. HIV-1 antibodies were detected in serum by using a commercial recombinant HIV-1 EIA (Abbott, Abbott Park, IL) and were confirmed by HIV-1 Western blot IgG assay. SI variants were detected by cocultivation of HIV-1-positive PBMC with MT2 cells [19]. Serum virus load was measured by use of a quantitative HIV-1 RNA nucleic acid sequence-based amplification (Organon Teknica, Boxtel, The Netherlands) [20]. Since 1989, CD4 cell counts have been determined by cytofluorometry.

Statistical analysis. Differences in HIV-1 disease progression between wild type CCR5 and CCR2b homozygotes and heterozygotes were studied using Kaplan-Meier product limit methods and the Cox proportional hazards analysis. Both the 1987 and the 1993 AIDS-defining criteria of the Centers for Disease Control and Prevention were used as outcomes; a first drop in CD4 cell count below 200 cells/ μ L was used as a separate outcome [21]. Since pre-AIDS mortality is high among IDUs, and, moreover, pre-AIDS death from natural causes is related to HIV-1 disease progression, pre-AIDS deaths from natural causes (i.e., not death by suicide, violence, or drug overdose), in addition to deaths from AIDS, were included as death outcomes [22]. An expected date of seroconversion was calculated for each IDU by use of the cohort-specific seroincidence distribution, conditional on the date of each subject's last HIV-1-negative and first positive test result [23, 24] (Geskus RB, personal communication). Event-free subjects were censored at the end of the study period (1 January 1997) or when they were lost to follow-up. Prospectively identified seroconverters were considered to be at risk from the estimated moment of seroconversion until the date of AIDS, death, or censoring. Retrospectively identified seroconverters entered the risk set at their first positive cohort visit (i.e., left truncation). Differences in survival between wild type homozygotes and heterozygotes were determined by the likelihood-ratio test. In the multivariate analysis, we adjusted for age, sex, and type of seroconverter (i.e., prospectively or retrospectively identified).

Since earlier studies reported that the protective effect of the CCR5 $\Delta 32/+$ and the CCR2b 64I/+ genotypes is stronger during the presence of only NSI variants [18, 25], we repeated our Cox analysis with a CD4 cell count <200/ μ L as an end point, while excluding trajectories during which SI variants of the virus were also present. Subjects were censored at the moment SI variants could be first detected.

Differences in virus load during the first 3 years after seroconversion between wild type genotypes and heterozygotes were compared, if available, each year (± 6 months) by use of the Mann-Whitney *U* test.

To further evaluate differences in HIV-1 disease progression be-

Table 1. Characteristics of study population of HIV-infected IDUs (*n* = 108).

Feature	No.	%
Prospective seroconverters	78	72
Retrospective seroconverters	30	28
Genotype distribution		
CCR5 wild type	86	80
+CCR2b wild type	68	63
+CCR2b 64I/+	18	17
CCR Δ32/+	22	20
+CCR2b wild type	19	18
+CCR2b 64I/+	3	3
Mean age (years)	32	
Sex		
Male	66	60
Female	42	40
White race	96	89
Median follow-up (years)	5.1	
Median year of seroconversion	1989	

tween the wild type CCR5 and CCR2b genotypes and mutant allele carriers, rates of CD4 cell decline were compared. Since the value of different CD4 cell measurements within each subject is related to previous measurements, a regression analysis for repeated measurement was done by use of a mixed linear model approach available in the statistical software package SAS (SAS, Inc., Cary, NC) [26]. In this model, each CD4 cell count depends on the value and the variation of the preceding CD4 cell count. Since the CD4 cell decline appeared not to be linear, the CD4 cell decline since seroconversion was modeled as a piecewise linear function. The change point between the pieces was identified as the time since the estimated moment of seroconversion that the change in slope was greatest. Therefore, the final model predicted two different rates of CD4 cell decline: a steep decline until ~6 months after seroconversion, followed by a slower decline from 6 months after sero-

conversion onward. Details on how this model was built have been described elsewhere [27, 28]. Inclusion criteria for subjects in this model were having had at least one CD4 cell count in a period of 3 months before seroconversion until 7 years after seroconversion, and having a seroconversion interval of <2 years. Under these criteria, 62 seroconverters were included. A total of 532 CD4 cell counts were used to build the model. Differences in patterns of CD4 cell decline between the CCR5 and CCR2b wild type homozygotes and CCR5 Δ32/+ and CCR2b 64I/+ heterozygotes were compared by use of the likelihood ratio test.

For the analysis of the case-control study, conditional logistic regression was used.

In general, *P* < .05 was considered statistically significant.

Results

CCR5 and CCR2b genotype distribution. Twenty-two (20%) of 108 seroconverters (95% confidence interval [CI], 12%–27%) were heterozygous for the CCR5 Δ32 mutation and 21 (19%) of 108 seroconverters (CI, 12%–28%) were heterozygous for the CCR2b 64I mutation. Only 3 subjects were heterozygous for both mutations. No persons homozygous for either the CCR5 Δ32 mutation or the CCR2b 64I mutation were identified. Other population characteristics are summarized in table 1.

Clinical course of HIV-1 infection in relation to CCR5 and CCR2b genotypes. Among the seroconverters, a total of 20 or 21 subjects developed AIDS, as determined by applying the 1987 and the 1993 AIDS-defining CDC criteria, respectively; after 8 years of follow-up, 80% (95% CI, 68%–88%) or 79% (65%–86%) were still AIDS-free (figure 1). CD4 cell counts of

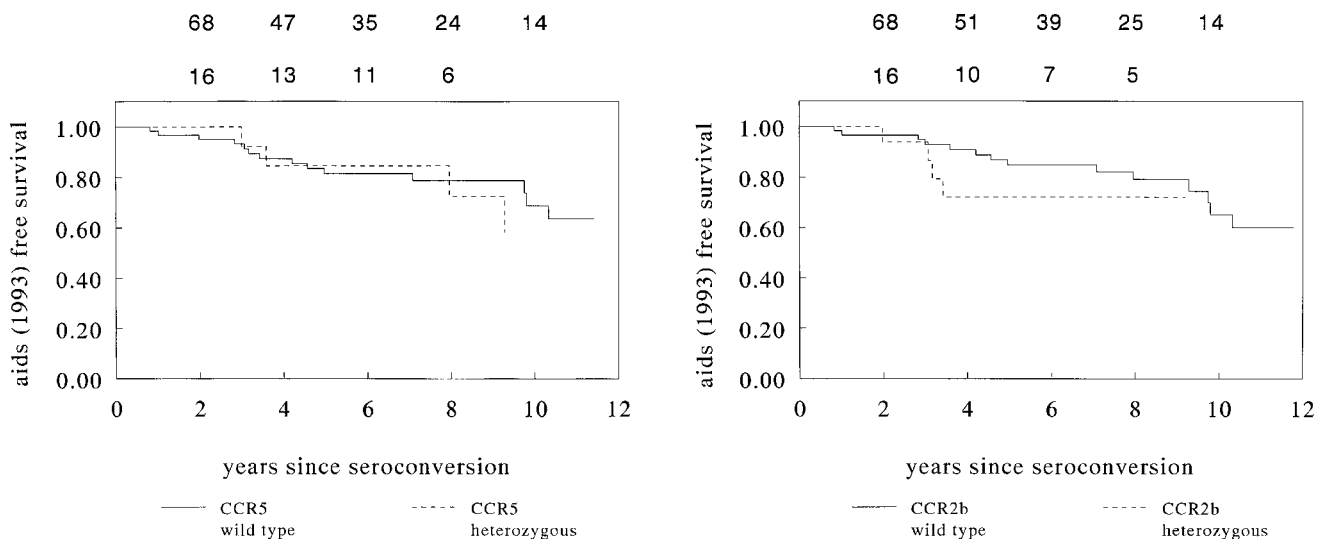


Figure 1. AIDS-free survival applying 1993 CDC AIDS definition for CCR5 wild type homozygotes and CCR5 Δ32 heterozygotes (left) and for CCR2b wild type homozygotes and CCR2b 64I heterozygotes (right). Numbers at top are numbers of wild type homozygotes and heterozygotes at risk, respectively; curves were truncated at <5 subjects at risk.

42 of the 107 subjects with available CD4 cell counts dropped below 200/ μ L. After 8 years of follow-up in 63% (50%–75%) of the IDUs, the CD4 cell count had not dropped below 200/ μ L. A total of 30 subjects died: 13 died of AIDS and another 7 died of a natural cause, resulting in 20 HIV-related deaths. In 2 subjects, the cause of death was not known; the other 8 deaths were not HIV-related. Within 8 years after seroconversion, 20% (12%–31%) of the IDUs had died of a natural cause.

When rates of disease progression between CCR5 wild type homozygotes and CCR5 Δ 32 heterozygotes were compared in a univariate Cox proportional hazard analysis, no significant protective effect of the CCR5 Δ 32/+ genotype was found. The relative hazard (RH) for progression to AIDS for heterozygotes was 1.5 (95% CI, 0.5–4.3) and 1.0 (0.4–3.2) for the 1987 and 1993 CDC definitions, respectively. RHs of 0.9 (0.3–2.6) and 1.1 (0.5–2.3) were found by using death and a drop in CD4 cell count below 200/ μ L as outcomes. Comparing rates of disease progression between CCR2b wild type homozygotes and CCR2b 64I heterozygotes in the same univariate Cox model did not reveal a significant protective effect of the CCR2b 64I heterozygous genotype (table 2). Adjusting for age, sex, and type of seroconverter (progressive or retrospective) did not change the RH substantially. Repeating our analysis while including only those subjects with a seroconversion interval <6 months ($n = 61$), with a CD4 cell count <200/ μ L as an end point, again we found no protective effect of the heterozygous CCR5 or CCR2b genotype on HIV-1 disease progression (RH, 1.2 [0.3–4.3] and 2.2 [0.6–8.4] for CCR5 Δ 32 and CCR2b 64I heterozygotes, respectively). Looking at the role of the CCR5 and CCR2b genotypes in HIV-1 disease progression while excluding trajectories in which SI variants were also present, with a CD4 cell count <200/ μ L as an end point, again did not reveal a significant difference between the groups of subjects with a wild type or heterozygous genotype (RH, 1.3 [0.6–3.0] and 1.6 [0.7–3.6] for CCR5 Δ 32 heterozygotes and CCR2b 64I heterozygotes, respectively).

We also compared differences in disease progression in relation to CCR5 and CCR2b genotypes by analyzing the CCR5 and CCR2b genotypes as compound genotypes—that is, com-

paring disease progression in subjects with the CCR5 Δ 32 and the CCR2b 64I heterozygous genotypes separately with subjects who are wild type homozygous for both CCR5 and CCR2b—with use of a CD4 cell count <200/ μ L as end point. Again, no protective effect for either the CCR5 or the CCR2b mutation was found: The RHs were 1.1 (0.3–3.9) and 1.1 (0.5–2.7) for CCR5 Δ 32 and CCR2b 64I heterozygotes, respectively. Adjusting for age, sex, and type of seroconverter (retrospective or prospective) again did not change these results substantially. Of the three dual heterozygotes, 1 died before having developed AIDS of a natural cause (endocarditis) after a follow-up of 5.2 years. The other 2 are still AIDS-free after a follow-up of 11.3 and 12.2 years, respectively. CD4 cell counts in all 3 subjects dropped below 200/ μ L.

In the case-control study, the vast majority of subjects who have remained AIDS-free for a long period of time—the long-term survivors—were not characterized by being heterozygous for the CCR5 or CCR2b mutation. Two (22%) of the 9 long-term survivors and 2 (12.5%) of the 16 progressors were heterozygous for the CCR5 Δ 32 mutation. One of the long-term survivors was heterozygous for the CCR2b 64I mutation (11%), compared with 3 (19%) of 16 progressors. The odds ratio for CCR5 Δ 32/+ heterozygotes to be long-term survivors was 1.4 (95% CI, 0.2–10.3), and that for CCR2b 64I/+ heterozygotes was 0.6 (0.05–7.4).

CD4 cell decline. Figure 2 shows the different rates of CD4 cell decline for the CCR5 and CCR2b wild type homozygotes and CCR5 Δ 32 and CCR2b 64I heterozygotes as predicted by the model. Mean baseline CD4 cell counts were 772 and 827/ μ L for the CCR5 wild type homozygotes and CCR5 Δ 32 heterozygotes, respectively, and 790 and 713/ μ L for the CCR2b wild type and 64I heterozygotes, both nonsignificant differences. The slopes that predict the CD4 cell decline from 6 months after seroconversion onward were not significantly different for the wild type and mutant CCR5 and CCR2b genotypes in a univariate and multivariate analysis ($P = .48$ and $P = .23$, respectively). Mean CD4 cell decline was 40 cells/ μ L/year for persons with a wild type CCR5 genotype and 54 cells/ μ L/year for CCR5 Δ 32/+ heterozygotes. For CCR2b wild type subjects, the mean predicted CD4 cell decline was 65 cells/ μ L/year.

Table 2. Relative hazards (RHs) and 95% confidence intervals (CIs) for the CCR5 Δ 32 genotype compared with CCR5 wild type genotype and CCR2b 64I/+ genotype compared with the CCR2b wild type genotype for different survival analysis end points.

	Events/ n	CCR5 Δ 32/+ genotype		CCR2b 64I/+ genotype	
		Crude RH (CI)	Adjusted RH ^a (CI)	Crude RH (CI)	Adjusted RH ^a (CI)
AIDS, 1987 definition	20/108	1.5 (0.5–4.3)	1.7 (0.6–4.8)	0.6 (0.2–2.1)	0.6 (0.2–2.2)
AIDS, 1993 definition	21/108	1.0 (0.4–3.2)	1.1 (0.4–3.3)	1.4 (0.5–4.0)	1.5 (0.5–4.4)
Death ^b	20/108	0.9 (0.3–2.6)	1.0 (0.3–3.3)	1.4 (0.6–3.8)	2.0 (0.7–5.8)
CD4 cell count <200/ μ L	42/107	1.1 (0.5–2.3)	1.2 (0.6–2.7)	1.5 (0.7–3.3)	1.2 (0.5–2.7)
CD4 cell count <200/ μ L and NSI trajectories ^c	30/96	1.3 (0.6–3.0)	1.4 (0.6–3.3)	1.6 (0.7–3.6)	1.2 (0.5–2.8)
CD4 cell count <200/ μ L and seroconversion interval <6 months	17/61	1.2 (0.3–4.3)	1.5 (0.4–5.3) ^d	2.2 (0.6–8.4)	1.7 (0.4–7.1) ^d

^a Adjusted for type of seroconverter, age, and sex.

^b By natural cause.

^c Censoring subjects after switch from non-syncytium-inducing (NSI) to SI.

^d Adjusted for sex and age.

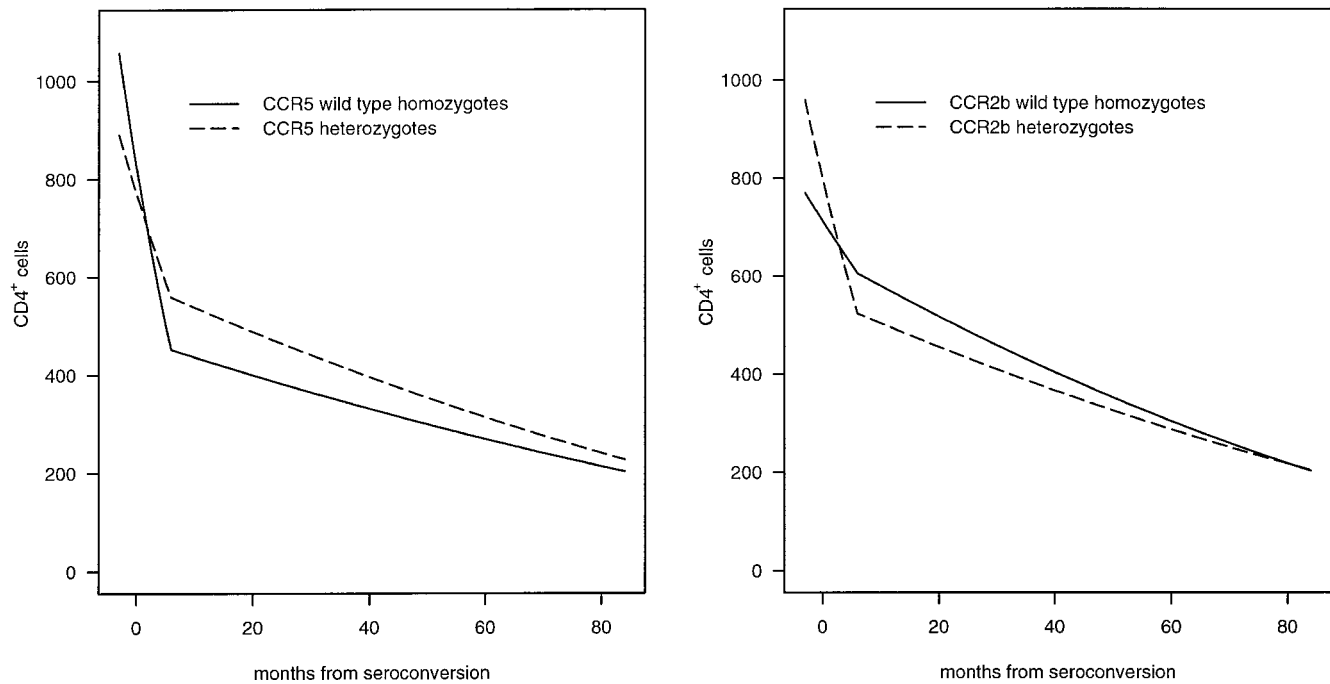


Figure 2. CD4 cell decline for CCR5 wild type homozygotes and CCR5 $\Delta 32$ heterozygotes (left) and for CCR2b wild type homozygotes and CCR2b 64I heterozygotes (right).

year, compared with 52 cells/ μ L/year for subjects heterozygous for the 64I mutation.

Serum virus load. No significant differences in median virus load at 1, 2, or 3 years (± 6 months) after seroconversion were observed between the groups of subjects with the CCR5 wild type or $\Delta 32/+$ genotype and the groups of subjects with the CCR2b wild type or CCR2b 64I/+ genotype. Both CCR5 $\Delta 32$ and CCR2b 64I heterozygotes tended to have a higher median serum virus load (table 3).

Discussion

We did not find evidence for slower disease progression in IDUs with a CCR5 $\Delta 32/+$ or CCR2b 64I/+ genotype. In the survival analysis, no significant difference in clinical course of HIV-1 infection was observed among 108 seroconverters. In fact, the RHs found in this study with AIDS, death, or a CD4 cell count $<200/\mu$ L as end points indicate a somewhat faster progression for subjects with a heterozygous CCR5 $\Delta 32$ or

CCR2b 64I genotype. The one exception to this phenomenon was an RH of 0.6 for subjects with a CCR2b 64I/+ genotype with AIDS according to the 1987 definition as an end point, which is difficult to account for but may be attributed to small numbers: Only 3 AIDS cases were present among CCR2b heterozygous subjects. Furthermore, long-term survivors were not more likely to be heterozygous for the mutant CCR5 and CCR2b genotypes than were progressors. The lack of effect of the mutant CCR5 and CCR2b genotypes on HIV disease progression was confirmed in the regression model we built for the rate of CD4 cell decline. Furthermore, no significant differences in serum virus load were observed between subjects with wild type and mutant CCR5 and CCR2b genotypes during the first 3 years after seroconversion. Thus, the observed lack of effect in our study of the CCR5 and CCR2b genotypes on HIV-1 disease progression was very consistent by application of different statistical methods and study designs.

The results of this study, however, are different from the results of several other studies, mainly done among homosexual men, that showed a protective effect of the CCR5 $\Delta 32$ and CCR2b 64I mutations on HIV-1 disease progression compared with subjects with a wild type genotype.

It is unlikely that the lack of effect found in this study is caused by major methodologic difficulties, since other markers, such as CD4 cell decline, high virus load, and occurrence of SI isolates, were significantly associated with disease progression in a time-dependent univariate analysis (data not shown).

Table 3. Median serum virus load (copies/mL) at 1, 2, and 3 years after seroconversion.

Year	CCR5			CCR2b		
	Wild type (n)	$\Delta 32$ (n)	P^a	Wild type (n)	64I/+ (n)	P^a
1	30.000 (39)	35.000 (8)	.9	29.500 (40)	33.000 (7)	.8
2	19.000 (31)	23.000 (7)	.7	18.000 (32)	38.500 (6)	.4
3	54.000 (21)	60.500 (6)	.9	26.000 (17)	76.000 (10)	.4

^a Mann-Whitney U test.

A possible explanation for the fact that no difference in disease progression between the wild type and mutant CCR5 and CCR2b genotypes was observed could be that the effect of the receptor genotype on HIV-1 disease progression is not very strong and that the relatively small sample size in our survival analysis failed to reveal this effect. However, a significant protective effect of the CCR5 $\Delta 32/+$ and CCR2b 64I/+ genotypes was observed in studies with an equally small sample size [14, 29]. No separate report on the role of CCR5 and CCR2b genotypes in HIV-1 disease progression in IDUs has been published thus far. In a study by Smith et al. [12] in which different cohorts of homosexual men, hemophiliacs, and IDUs were analyzed together, a protective effect of the mutant CCR5 and CCR2b genotypes was found for the combined cohorts. When the RHs for HIV-1 disease progression for the separate cohorts were compared, however, no association was found between CCR2b genotype in the ALIVE cohort of IDUs. One of the explanations for this phenomenon suggested by the authors is the different racial background of this cohort, which is mainly composed of African Americans. However, among IDUs of the French SEROCO and SEROGEST cohorts, both seroconverters and seroprevalent subjects, no association was found between CCR5 genotype and HIV-1 disease progression when AIDS according to the 1993 CDC AIDS-defining criteria was used as an outcome (Meyer L, personal communication).

Therefore, to explain the lack of the protective effect of the mutant CCR5 and CCR2b genotypes found in this study, other possible explanations than lack of power or methodologic shortcomings need to be explored. One possible explanation could be that levels of β -chemokines, which inhibit HIV-1 replication in vitro [30, 31], are higher in IDUs, perhaps as a response to exposure to many different pathogens. This would be in agreement with the observation that subjects who frequently borrow injection equipment have a slower rate of progression [32]. In vitro experiments have shown that ligation of the CCR5 or CCR2b receptor with its chemokines down-regulates receptor expression [33, 34]. It may be that in IDUs, because of higher chemokine levels or other unknown mechanisms, receptor down-regulation occurs. If that were the case, the receptor genotype does not significantly contribute to the level of expression of functional CCR5 and CCR2b receptors and therefore has no differential effect on HIV-1 disease progression. Alternatively, even without receptor down-regulation, higher levels of circulating β -chemokines could diminish the effect of receptor genotype on the rate of HIV-1 replication. If these hypotheses were true, one would expect the clinical course of HIV-1 infection to be more benign in IDUs. Although this has been reported by some groups [35, 36], we did not find evidence for this in our own studies [37, 38].

Another explanation could be that the virus population among IDUs is less dependent on CCR5 or CCR2b as a coreceptor for entry into CD4 cells, perhaps because of use of other receptors. Indeed, in The Netherlands and the United

States, intravenous drug use-associated HIV-1 strains can be distinguished from strains circulating among homosexual men by differences in the sequence of the V3 loop [39], the HIV-1 envelope region involved in determining viral tropism and coreceptor usage and possibly directly interacting with the coreceptor. The hypothesis that virus variants among IDUs can use other receptors than CCR5 or CCR2b is supported by the fact that switches in SI status occur less frequently among IDUs than among homosexual men [16], indicating that the selection pressure toward SI switch among IDUs is less strong, possibly because virus variants among IDUs can use other coreceptors.

Finally, the different transmission route in IDUs, in whom the initial viral replication is located in lymph nodes instead of in mucosal macrophages after sexual transmission, could trigger a host immune response that involves a less critical role for the CCR5 and CCR2b genotypes in disease progression. This is in agreement with the fact that Dean et al. [7] did not find a greater percentage of heterozygotes among long-term non-progressors in the hemophilia cohorts. However, in a survival analysis of the Multicenter Hemophilia Cohort Study, Smith et al. [12] did find a protective effect of the CCR5 and CCR2b heterozygous genotypes on HIV-1 disease progression, suggesting that difference in transmission route is a less plausible explanation.

Other longitudinal studies on the effect of CCR5 and CCR2b genotypes on HIV-1 disease progression need to confirm the results of our study. We are currently investigating the above-postulated explanations for the absence of a protective effect of the mutant CCR5 and CCR2b genotypes on HIV-1 disease progression in our cohort of HIV-1 infected IDUs.

Acknowledgments

We thank J. Bax, A. Snuverink, I. Spijkerman, and H. Fennema for data collection; M. Prins, E. J. C. van Ameijden, A. M. de Roda Husman, and R. P. van Rij for carefully reading and discussing the manuscript; and J. Goudsmit and F. De Wolf of the Academic Medical Center, Amsterdam, for performing the virologic assays.

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