



## UvA-DARE (Digital Academic Repository)

### CC chemokine receptor 5 delta32 and CC chemokine receptor 2 64I polymorphisms do not influence the virologic and immunologic response to antiretroviral combination therapy in human immunodeficiency virus type 1-infected patients

Wit, F.W.N.M.; van Rij, R.P.; Weverling, G.J.; Lange, J.M.A.; Schuitemaker, H.

**DOI**

[10.1086/345677](https://doi.org/10.1086/345677)

**Publication date**

2002

**Published in**

The Journal of Infectious Diseases

[Link to publication](#)

**Citation for published version (APA):**

Wit, F. W. N. M., van Rij, R. P., Weverling, G. J., Lange, J. M. A., & Schuitemaker, H. (2002). CC chemokine receptor 5 delta32 and CC chemokine receptor 2 64I polymorphisms do not influence the virologic and immunologic response to antiretroviral combination therapy in human immunodeficiency virus type 1-infected patients. *The Journal of Infectious Diseases*, 186, 1726-1732. <https://doi.org/10.1086/345677>

**General rights**

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

**Disclaimer/Complaints regulations**

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (<https://dare.uva.nl>)

## CC Chemokine Receptor 5 $\Delta$ 32 and CC Chemokine Receptor 2 64I Polymorphisms Do Not Influence the Virologic and Immunologic Response to Antiretroviral Combination Therapy in Human Immunodeficiency Virus Type 1–Infected Patients

Ferdinand W. N. M. Wit,<sup>1</sup> Ronald P. van Rij,<sup>2</sup>  
Gerrit Jan Weverling,<sup>3</sup> Joep M. A. Lange,<sup>1,4</sup>  
and Hanneke Schuitemaker<sup>2</sup>

<sup>1</sup>Department of Internal Medicine, Division of Infectious Diseases, Tropical Medicine, and AIDS, National AIDS Therapy Evaluation Centre, <sup>2</sup>Sanquin Research at CLB and Landsteiner Laboratory, and Departments of <sup>3</sup>Clinical Epidemiology and Biostatistics and <sup>4</sup>Human Retrovirology, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

To investigate the influence of the CC chemokine receptor 2 64I and CC chemokine receptor 5  $\Delta$ 32 polymorphisms on the virologic and immunologic response of human immunodeficiency virus type 1 (HIV-1)–infected patients to highly active antiretroviral therapy, data from 4 clinical studies were pooled. The prevalence of the CCR5  $\Delta$ 32 polymorphism was 21% (27 of 130 subjects), and the prevalence of the CCR2 64I polymorphism was 15% (19 of 130 subjects). There were no major differences between subjects with and without polymorphisms in the CCR5 and/or CCR2 genes with respect to the rate of initial viral clearance, proportion of subjects with plasma HIV-1 RNA levels below the lower limit of quantification, rate of virologic treatment failure, immunologic responses, and disease progression during 96 weeks of follow-up.

Human immunodeficiency virus type 1 (HIV-1) infects cells expressing the CD4 receptor on their surfaces. In 1996, it was recognized that macrophage-tropic strains of HIV-1 also need CCR5 as a coreceptor to gain entry into host cells [1, 2]. A 32-bp deletion in the CCR5 gene (CCR5  $\Delta$ 32) results in a reduced expression of CCR5 on the cell surface [3]. Persons homozygous for the CCR5  $\Delta$ 32 polymorphism are almost completely protected against HIV-1 infection [4, 5]. Persons heterozygous for the CCR5  $\Delta$ 32 polymorphism have a slower progression from infection to AIDS [4, 6–10]. The 64I polymorphism in the gene for CCR2, a minor coreceptor for HIV, is not associated with protection against HIV-1 infection, but both heterozygotes and homozygotes for the CCR2 64I polymorphism progress more slowly from infection to AIDS [10, 11]. The CCR2 64I polymorphism is in complete linkage disequilibrium with a single-nucleotide polymorphism in the promoter region of the CCR5

gene (C927T) that has been associated with a slower HIV-1 disease progression [11, 12].

Some studies report that CCR2 and/or CCR5 polymorphisms affect the virologic and immunologic treatment response to antiretroviral therapy [13–15], but others have found no significant differences in the response to treatment of individuals with these polymorphisms and the response of those without [16–18]. In this study, we retrospectively investigated the influence of the CCR2 64I and CCR5  $\Delta$ 32 polymorphisms on the virologic and immunologic response to highly active antiretroviral therapy (HAART).

### Subjects, Materials, and Methods

**Subjects.** In this study, we included participants from several published studies in whom CCR2 and CCR5 genotypes could be determined retrospectively in frozen peripheral blood mononuclear cells (PBMC): 29 participants from the Amsterdam Duration of Antiretroviral Medication (ADAM) study [19, 20], 44 from the Bristol-Myers Squibb AI455-050 (BMS050) protocol [21, 22], 47 from the Amsterdam Cohort Studies on AIDS [23], and 10 from the ERA study [24]. These studies were chosen because of the availability of frozen PBMC for CCR2 and CCR5 genotyping and because the patients were treated with  $\geq 3$  antiretroviral drugs. Because the individual studies had insufficient patient numbers for the patient groups to be analyzed separately, we pooled the data to increase statistical power. Detailed descriptions of the ADAM, BMS050, and ERA studies has been published elsewhere [19–22, 24]. Participants in the Amsterdam Cohort Studies on AIDS initiated antiretroviral therapy as directed by their treating physicians, who followed the treatment guidelines of the time. These patients

Received 29 April 2002; revised 5 September 2002; electronically published 22 November 2002.

All patients gave written informed consent before entering the original studies and gave consent for storing materials for later studies of any type.

Financial support: Dutch AIDS fund (grant 1305); Netherlands Organization for Scientific Research. Some of this study was performed as part of the Amsterdam Cohort Studies on AIDS, a collaboration of the Municipal Health Service, Academic Medical Center, and Sanquin Research at CLB.

Reprints or correspondence: Dr. Ferdinand W. N. M. Wit, National AIDS Therapy Evaluation Center, Academic Medical Centre, Rm. T0-120, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands (f.w.wit@amc.uva.nl).

The Journal of Infectious Diseases 2002;186:1726–32

© 2002 by the Infectious Diseases Society of America. All rights reserved.  
0022-1899/2002/18612-0005\$15.00

were not treated with a standardized antiretroviral combination regimen but with the regimen that the treating physicians deemed most suitable for each subject. Only subjects who had no prior exposure to antiretroviral agents before the initiation of HAART were included in our analysis.

**Laboratory measurements.** Nucleic acids were extracted from cryopreserved PBMC by use of a blood kit (Qiagen). The CCR2 genotype was determined using polymerase chain reaction (PCR)–restriction fragment–length polymorphism analysis [25]. The CCR5 genotype was determined using a PCR-based method [9]. Methods used for measurement of plasma HIV load (pVL) varied by study and are described elsewhere [19–24]. For the purposes of analysis, the lower limit of quantification (LLQ) of the ultrasensitive assays was set to 400 copies/mL. CD4<sup>+</sup> and CD8<sup>+</sup> T cell numbers were analyzed by flow cytometry.

**Statistical analysis.** The subjects were grouped by CCR5 genotype. Data were analyzed according to the intent-to-treat principle. All patients had a potential follow-up of  $\geq 96$  weeks after the start of antiretroviral therapy. Data were censored 96 weeks after the start of antiretroviral therapy.

Baseline demographic characteristics and antiretroviral medications used were tabulated and compared among patients with different genotypes. The rate of initial viral clearance was determined by use of Kaplan-Meier estimates of the time between the initiation of HAART and measurement of the first pVL that was below the LLQ. The effect of CCR2 and CCR5 genotype on this interval was investigated by use of a multivariate proportional hazards model, adjusted for parameters that were statistically significantly associated with the outcome. The investigated parameters were baseline pVL, baseline CD4<sup>+</sup> cell count, sex, age, mode of HIV transmission, years of documented HIV infection, use of antiretroviral medication (i.e., number of antiretroviral agents used), and Centers for Disease Control and Prevention (CDC) HIV infection stage [26]. The proportion of subjects with a pVL below LLQ during the first 96 weeks of follow-up was plotted for each genotype and compared at weeks 48 and 96.

Virologic treatment failure was defined as either failure to suppress the pVL to below the LLQ within 24 weeks after the start of antiretroviral therapy or as measurement of a single pVL  $>5000$  copies/mL in a patient in whom a pVL below the LLQ had been found. Time to virologic treatment failure was estimated by use of Kaplan-Meier estimates. We used a multivariate proportional hazards model to investigate the effect of CCR2 and CCR5 genotype on the time to virologic treatment failure, adjusted for parameters that were statistically significantly associated with the outcome. The investigated parameters were baseline pVL, baseline CD4<sup>+</sup> cell count, sex, age, mode of HIV transmission, years of documented HIV infection, antiretroviral medication use (i.e., number of antiretroviral agents used), and CDC stage. The CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses of patients with each genotype were modeled for the first 96 weeks of follow-up. These analyses were repeated for the subjects grouped by CCR2 genotype.

Data were analyzed with SAS software (version 8.02; SAS Institute). Group comparisons were made using Student's *t* test or the Wilcoxon rank sum test for continuous data and the  $\chi^2$  test or Fisher's exact test for categorical data. Kaplan-Meier estimates were compared using the log-rank test. CD4<sup>+</sup> and CD8<sup>+</sup> cell count responses were modeled using PROC MIXED from SAS, which

accommodates repeated measurements and estimates mean values by a least-squares analysis. Differences between groups were considered to be statistically significant at  $P < .05$ . All *P* values reported are 2-sided.

## Results

**Baseline demographic characteristics in relation to the CCR5 and CCR2 genotypes.** Of 130 subjects, 103 were homozygous for the CCR5 wild-type allele, and 27 were heterozygous for the CCR5  $\Delta 32$  polymorphism. No subjects were homozygous for the CCR5  $\Delta 32$  polymorphism. One hundred eleven subjects were homozygous for the CCR2 wild-type allele, and 17 subjects were heterozygous and 2 homozygous for the CCR2 64I polymorphism. Five subjects were heterozygous for both the CCR5  $\Delta 32$  and the CCR2 64I polymorphism. None of the subjects who were homozygous for the CCR2 64I polymorphism carried the CCR5  $\Delta 32$  polymorphism. Because the number of subjects who were homozygous for the CCR2 64I polymorphism was small, CCR2 64I heterozygotes and homozygotes were analyzed as a single group.

The duration of documented HIV seropositivity at the time that antiretroviral therapy was initiated was significantly longer among subjects with the CCR5  $\Delta 32$  polymorphism (table 1). Subjects with the CCR2 64I polymorphism had significantly higher CD4<sup>+</sup> cell counts, tended to have had fewer AIDS-defining illnesses, and were more often female than male. There were no significant differences between subjects with and subjects without the CCR5  $\Delta 32$  polymorphism and between subjects with and subjects without the CCR2 64I polymorphism with regard to initial antiretroviral regimens (table 2).

**Virologic responses to antiretroviral therapy in relation to the CCR5 and CCR2 genotypes.** Figure 1A and 1B show the time to first pVL below the LLQ. There were no statistically significant differences between the CCR5 genotype groups ( $P = .23$ ; log-rank test) or between the CCR2 genotype groups ( $P = .59$ ; log-rank test). In a multivariate proportional hazards model, the number of drugs used in the first antiretroviral regimen, the duration of HIV infection, and baseline pVL were statistically significantly associated with the time to the first pVL below the LLQ. After the analysis was adjusted for these parameters, neither the CCR2 nor the CCR5 genotypes were associated with time to the first pVL below the LLQ. Hazard ratios and 95% confidence intervals (CIs) were 1.15 (95% CI, 0.70–1.91;  $P = .58$ ) for the CCR2 genotypes and 1.14 (95% CI, 0.73–1.78;  $P = .55$ ) for the CCR5 genotypes.

Figure 1C and 1D show the proportion of subjects with pVLs below the LLQ. There were no statistically significant differences among the genotype groups. Figure 1E and 1F show the time to virologic failure. There were no statistically significant differences between the CCR5 genotype groups ( $P = .28$ ; log-rank test) and between the CCR2 genotype groups ( $P = .42$ ; log-rank test). Two other definitions of virologic failure (pVL

**Table 1.** Baseline demographic characteristics of human immunodeficiency virus type 1 (HIV-1)-infected patients included in a study of the influence of the CCR5  $\Delta 32$  and CCR2 64I polymorphisms on virologic and immunologic responses to highly active antiretroviral therapy.

Variable	CCR5 genotype			CCR2 genotype		
	wt	$\Delta 32^a$	P	wt	64I <sup>b</sup>	P
No. (%) of subjects						
Total	103 (79)	27 (21)		111 (85)	19 (15)	
From study						
BMS050	33 (75)	11 (25)	.70	35 (80)	9 (20)	.54
ADAM	25 (86)	4 (14)		24 (83)	5 (17)	
ERA	8 (80)	2 (20)		9 (90)	1 (10)	
Amsterdam Cohort Studies on AIDS	37 (79)	10 (21)		43 (91)	4 (9)	
Sex, no. (%) male	96 (93)	27 (100)	.34	107 (96)	16 (84)	.064
Age, median years (IQR)	37 (32–46)	40 (35–46)	.23	38 (32–46)	41 (32–47)	.93
Transmission category, no. (%) of subjects						
Men having sex with men	78 (76)	20 (74)	.53	85 (77)	13 (68)	.27
Unknown	12 (12)	6 (22)		16 (14)	2 (11)	
Intravenous drug use	6 (6)	1 (4)		4 (4)	3 (16)	
Heterosexual	6 (6)	0		5 (5)	1 (5)	
Other	1 (1)	0		1 (1)	0	
Documented HIV seropositivity, median years (IQR)	3.4 (0.7–6.4)	7.2 (0.8–10.3)	.038	3.5 (0.6–6.7)	4.5 (0.9–9.9)	.25
CDC stage, <sup>c</sup> no. (%) of subjects						
A	66 (64)	19 (70)	.83	75 (68)	10 (53)	.078
B	28 (27)	7 (26)		26 (23)	9 (47)	
C	9 (9)	1 (4)		10 (9)	0	
Plasma HIV RNA load, median log <sub>10</sub> copies/mL (IQR)	4.7 (4.3–5.0)	4.5 (4.3–4.8)	.27	4.6 (4.3–5.0)	4.5 (4.1–4.9)	.29
CD4 <sup>+</sup> T cell count, mean cells/mm <sup>3</sup> (SD)	347 (181)	347 (190)	.99	334 (182)	422 (167)	.051
CD8 <sup>+</sup> T cell count, mean cells/mm <sup>3</sup> (SD)	1097 (594)	1203 (491)	.39	1110 (581)	1171 (544)	.67

NOTE. ADAM, Amsterdam Duration of Antiretroviral Medication; BMS050, Bristol-Myers Squibb AI455-050; IQR, interquartile range; wt, wild type.

<sup>a</sup> Heterozygous for the  $\Delta 32$  polymorphism in the CCR5 gene.

<sup>b</sup> Heterozygous or homozygous for the 64I polymorphism in the CCR2 gene.

<sup>c</sup> HIV infection stages are from the Centers for Disease Control and Prevention (CDC) classification [26].

>1000 and pVL >2000 copies/mL) yielded slightly higher failure rates but, again, without significant differences between the CCR2 groups and between the CCR5 groups (data not shown). In a multivariate proportional hazards model, the number of drugs used in the first antiretroviral regimen was statistically significantly associated with time to virologic treatment failure. When we adjusted the analysis for the number of drugs used in the first antiretroviral regimen, the CCR2 (hazard ratio, 1.4; 95% CI, 0.6–3.5;  $P = .47$ ) and CCR5 (hazard ratio, 1.4; 95% CI, 0.6–3.1;  $P = .44$ ) genotypes were not associated with the time to virologic treatment failure.

*Immunologic response to antiretroviral therapy in relation to the CCR5 and CCR2 genotypes.* Figure 2A and 2B show the modeled mean CD4<sup>+</sup> cell count during the first 96 weeks of follow-up. There were no significant differences between subjects with and subjects without the CCR5  $\Delta 32$  polymorphism. After 96 weeks, the mean CD4<sup>+</sup> cell count was 580 cells/mm<sup>3</sup> for subjects homozygous for the CCR5 wild-type allele and 571 cells/mm<sup>3</sup> for subjects heterozygous for the CCR5  $\Delta 32$  polymorphism. Subjects with the CCR2 64I polymorphism had a higher mean CD4<sup>+</sup> cell count at baseline than did subjects with the CCR2 wild-type allele (422 vs. 334 cells/mm<sup>3</sup>). After 96 weeks of therapy, the mean CD4<sup>+</sup> cell counts did not differ significantly between these 2 groups (578 cells/mm<sup>3</sup> for CCR2

wild-type homozygotes and 585 cells/mm<sup>3</sup> for subjects with the CCR2 64I polymorphism). The smaller increase that was seen among subjects with the CCR2 64I polymorphism did not reach statistical significance ( $P = .11$ ).

Figure 2C and 2D show the modeled mean CD8<sup>+</sup> cell count

**Table 2.** Antiretroviral treatment regimens, shown in order of potency, for human immunodeficiency virus type 1 (HIV-1)-infected patients included in a study of the influence of the CCR5  $\Delta 32$  and CCR2 64I polymorphisms on virologic and immunologic responses to highly active antiretroviral therapy.

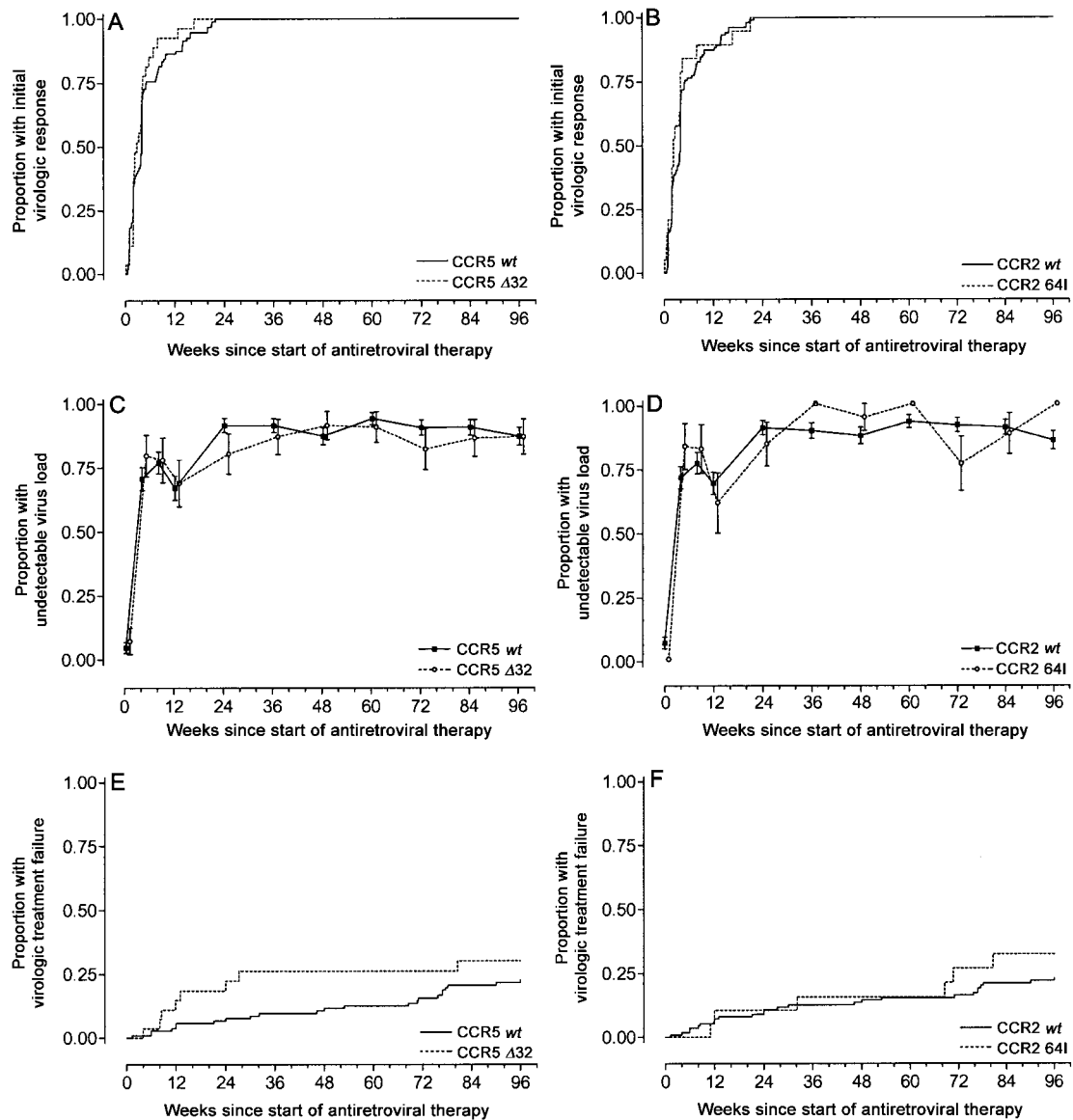
Regimen	No. (%) of subjects with CCR5 genotype		No. (%) of subjects with CCR2 genotype	
	wt	$\Delta 32^a$	wt	64I <sup>b</sup>
3 NRTI + 1 NNRTI + 1 PI	8 (8)	2 (7)	9 (8)	1 (5)
2 NRTI + 2 PI	25 (24)	4 (15)	24 (22)	5 (26)
2 NRTI + 1 PI	37 (36)	10 (37)	43 (39)	4 (21)
2 NRTI <sup>c</sup>	33 (32)	11 (41)	35 (31)	9 (48)

NOTE. NRTI, nucleoside analogue reverse-transcriptase inhibitor; NNRTI, nonnucleoside analogue reverse-transcriptase inhibitor; PI, protease inhibitor; wt, wild type.

<sup>a</sup> Heterozygous for the  $\Delta 32$  polymorphism in the CCR5 gene.

<sup>b</sup> Heterozygous or homozygous for the 64I polymorphism in the CCR2 gene.

<sup>c</sup> This is the initial regimen used in the Bristol-Myers Squibb AI455-050 protocol; indinavir was added to the regimen after 12 weeks.

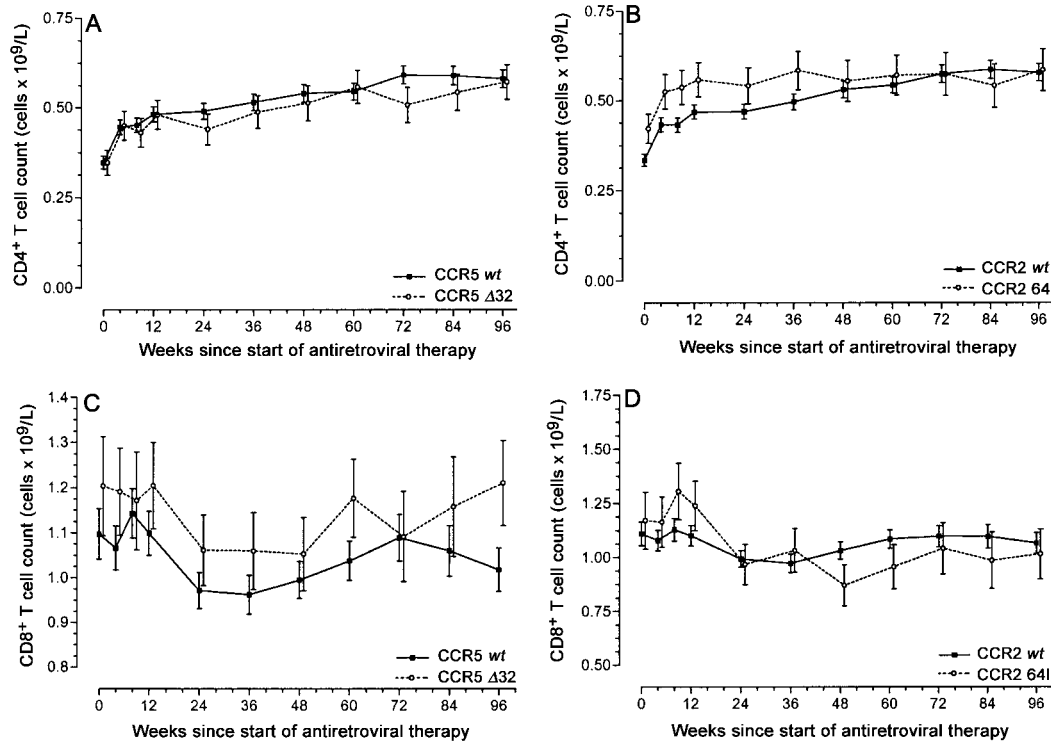


**Figure 1.** Virologic response to antiretroviral combination therapy among human immunodeficiency virus type 1 (HIV-1)-infected patients. *A, C, and E,* Data from subjects homozygous for the CCR5 wild-type (*wt*) allele and subjects heterozygous for the CCR5  $\Delta 32$  polymorphism. *B, D, and F,* Data from subjects homozygous for the CCR2 *wt* allele and heterozygous or homozygous for the CCR2 64I polymorphism. *A and B,* Kaplan-Meier estimates of the time to first plasma HIV-1 RNA measurement below the lower limit of quantification (LLQ). *C and D,* Proportion of subjects with plasma HIV-1 RNA measurements below the LLQ. *E and F,* Kaplan-Meier estimates of the time to virologic failure.

during the first 96 weeks of follow-up. There were no significant differences between subjects with and subjects without the CCR5  $\Delta 32$  polymorphism. Among the subjects who were homozygous for the CCR2 wild-type allele, the mean CD8<sup>+</sup> cell count did not change significantly during the 96 weeks of follow-up. There was a significant decrease in the mean CD8<sup>+</sup> cell count among subjects with the CCR2 64I polymorphism (*P* = .011).

*Post hoc analysis.* Because no differences were observed between patients with and patients without the CCR2 64I poly-

morphism and between patients with and patients without the CCR5  $\Delta 32$  polymorphism, we repeated the analyses described above, using 2 other classification schemes defined by the combined CCR2 and CCR5 genotypes of the patients. In the first classification, we divided the patients into 4 groups: 64I<sup>-</sup>/ $\Delta 32$ <sup>-</sup> (*n* = 89), 64I<sup>+</sup>/ $\Delta 32$ <sup>-</sup> (*n* = 14), 64I<sup>-</sup>/ $\Delta 32$ <sup>+</sup> (*n* = 22), and 64I<sup>+</sup>/ $\Delta 32$ <sup>+</sup> (*n* = 5). In the second classification scheme, we divided the patients into 2 groups: a group homozygous for both the wild-type CCR2 allele and the wild-type CCR5 allele (*n* = 89)



**Figure 2.** Immunologic response to antiretroviral combination therapy among human immunodeficiency virus type 1–infected patients. *A* and *C*, Data from subjects homozygous for the CCR5 wild-type (*wt*) allele and subjects heterozygous for the CCR5 Δ32 polymorphism. *B* and *D*, Data from subjects homozygous for the CCR2 *wt* allele and subjects heterozygous or homozygous for the CCR2 64I polymorphism. Changes in CD4<sup>+</sup> (*A* and *B*) and CD8<sup>+</sup> (*C* and *D*) T cell counts from baseline are shown.

and a group with the CCR2 64I polymorphism, the CCR5 Δ32 polymorphism, or both ( $n = 41$ ). For all repeated analyses that used both classification schemes, the results closely matched those of the prior analyses. There were no statistically significant differences between the groups (data not shown).

## Discussion

We studied the influence of CCR5 and CCR2 polymorphisms on virologic and immunologic responses to first-line antiretroviral combination therapy in 130 HIV-1–infected adults, predominantly white men. The prevalences of the CCR5 Δ32 and CCR2 64I polymorphisms were 21% and 15%, respectively, which is in accordance with the findings of previous reports [13, 14, 16–18, 27]. The duration of documented HIV seropositivity before initiation of antiretroviral therapy was longer among patients who were heterozygous for the CCR5 Δ32 polymorphism than among patients with the wild-type CCR5 genotype, even though these groups had similar CD4<sup>+</sup> cell counts. This finding is in agreement with those of other studies, in which patients who were heterozygous for the CCR5 Δ32 polymorphism had slower disease progression than patients without the polymorphism [4, 6–10]. We found no differences between subjects with and subjects without polymorphisms in the CCR5

and/or CCR2 genes with respect to the rate of initial viral clearance, the proportion of subjects with pVLs below the LLQ, and the rate of virologic treatment failure during a 96-week follow-up period. Subjects with and subjects without the CCR5 Δ32 polymorphism had similar patterns in CD4<sup>+</sup> and CD8<sup>+</sup> cell counts during the 96 weeks of follow-up.

In several previous studies, the CCR5 Δ32 heterozygous genotype was associated with an improved treatment response among treatment-naïve patients but not among treatment-experienced subjects [13–15]; however, others found no significant differences [16–18]. Yamashita et al. [13] observed 397 patients for 33 months after the initiation of HAART and found that a small subgroup of patients who were heterozygous for the CCR5 Δ32 genotype and had baseline CD4<sup>+</sup> cell counts >400 cells/mm<sup>3</sup> had a better short-term immunologic response. This short-term immunologic effect was not observed for the total patient group, and the virologic and long-term immunologic responses were not statistically different from those among patients who were homozygous for the CCR5 wild-type allele.

Guérin et al. [14] observed 166 patients for 1 year and found higher virologic and immunologic response rates among patients who were heterozygous for the CCR5 Δ32 genotype. However, their definitions of these responses differed from the definitions we used. Virologic success was defined as  $\geq 1$  finding

of a pVL below the limit of detection within 6 months of initiation of therapy. All but 1 patient in our study, but only 63% of those studied by Gu erin et al. [14], had virologic responses according to this definition (figure 1A and 1B), which suggests that viral replication in general was better suppressed in our study than in the study by Gu erin et al. [14].

Valdez et al. [15] observed 113 patients for ~1 year after the initiation of HAART and found higher virologic and immunologic response rates among patients who were heterozygous for the CCR5  $\Delta$ 32 polymorphism. In that study, virologic success was defined as a finding of a pVL <400 copies/mL at the last clinical visit (mean follow-up, ~45 weeks). Of the patients studied, 61% experienced virologic success (in our study, ~90% had virologic success, according to this definition; figure 1C and 1D). Furthermore, the study by Valdez et al. only included therapy-adherent white persons, which limited the generalizability of their results.

O'Brien et al. [16] studied the effect of CCR2 and CCR5 gene polymorphisms in 272 patients and found no significant differences for the primary end points, time to initial suppression and suppression failure. They found a significantly smaller reduction in the mean pVL after 24 weeks of therapy in a small subgroup of 25 patients who were homozygous for the CCR5-59029 A allele. No differences were observed in suppression among CCR5  $\Delta$ 32 heterozygotes at this time point. Furthermore, very strict inclusion criteria were used that limited the analysis to 272 white non-Hispanic patients who completed  $\geq$ 16 weeks of therapy.

Brumme et al. [17] and Bratt et al. [18] investigated the influence of the CCR5  $\Delta$ 32 polymorphism in 436 and 147 patients, respectively, and both found no differences in treatment response. Like our analysis, these studies used an intent-to-treat approach and did not exclude any patients from the analysis. Overall differences in study design, sample size, and efficacy of HAART regimens have made it difficult to compare findings from various cohorts.

Our study has several limitations. The sample size was relatively small, which limits the power to detect small differences between the patients with and patients without CCR2 and CCR5 polymorphisms and makes our findings more susceptible to chance fluctuations. Because we do not have adherence data on all patients, we cannot exclude the possibility that random differences in adherence contributed to the observed results.

The CCR5  $\Delta$ 32 polymorphism has been associated with a reduced percentage of CCR5-expressing cells among CD4<sup>+</sup> cells and, consequently, with a reduced number of potential HIV target cells [3]. This may be the direct explanation for the lower initial pVL after acute HIV-1 infection and slower progression toward symptomatic disease that are associated with the CCR5  $\Delta$ 32 heterozygous genotype [4, 6–8, 10]. Typically, antiretroviral therapy is started relatively late in the course of HIV-1 infection, when the generalized immune activation has resulted in an up-regulation of the expression of CCR5 on lymphocytes, possibly

canceling out any benefits associated with the CCR5  $\Delta$ 32 polymorphism.

Evolving viral coreceptor use is another possible reason for the observed lack of influence of the CCR5  $\Delta$ 32 polymorphism on treatment response. CXCR4-using HIV-1 variants emerge in approximately one-half of HIV-1-infected persons, in general, when CD4<sup>+</sup> cell counts decrease to <500 cells/mm<sup>3</sup> [28]. Because the mean CD4<sup>+</sup> cell count in our study population was <400 cells/mm<sup>3</sup>, it is likely that a significant proportion of the patients carried CXCR4-using variants at baseline. The finding that the favorable effect of the CCR5  $\Delta$ 32 polymorphism on the natural history of HIV-1 disease is most clearly evident before the development of AIDS [10] and less pronounced when CXCR4-using variants are present [9, 29] may explain the lack of a protective effect of CCR5  $\Delta$ 32 on therapy response in our study population.

It is intriguing that, in the studies in which the most pronounced beneficial effects of CCR5  $\Delta$ 32 on therapy response were reported [14, 15], the percentage of patients successfully suppressing viral replication seemed to be lower than that in our study population. It may be that when less-potent antiretroviral therapy is used, the number of available target cells (as influenced by the CCR5  $\Delta$ 32 polymorphism) influences the extent of ongoing viral replication and, consequently, the immunologic and virologic response to therapy. Although ongoing low-level viral replication during optimal suppressive therapy has been demonstrated by several methods [30–34], our results and those of others [17, 18] suggest that the number of CCR5-positive target cells does not influence the ongoing viral replication when therapy is more optimal.

In conclusion, we found no evidence that the CCR5  $\Delta$ 32 and CCR2 64I polymorphisms have a significant influence on short- or long-term virologic and immunologic responses to antiretroviral combination therapy in adult subjects without prior exposure to antiretroviral drugs. Because the scientific literature on this topic now contains conflicting findings, we propose to perform a meta-analysis that uses all available data from previously published studies and from groups with relevant unpublished data.

#### Acknowledgments

We thank all the investigators involved in the Amsterdam Duration of Antiretroviral Medication, Bristol-Myers Squibb AI455-050, and ERA studies and the Amsterdam Cohort Studies on AIDS and all patients who participated in these studies.

#### References

1. Deng H, Liu R, Ellmeier W, et al. Identification of a major co-receptor for primary isolates of HIV-1. *Nature* **1996**;381:661–6.
2. Dragic T, Litwin V, Allaway GP, et al. HIV-1 entry into CD4<sup>+</sup> cells is mediated by the chemokine receptor CC-CKR-5. *Nature* **1996**;381:667–73.
3. Benkirane M, Jin DY, Chun RF, Koup RA, Jeang KT. Mechanism of trans-

- dominant inhibition of CCR5-mediated HIV-1 infection by *ccr5Δ32*. *J Biol Chem* **1997**;272:30603–6.
4. Huang Y, Paxton WA, Wolinsky SM, et al. The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. *Nat Med* **1996**;2:1240–3.
  5. Liu R, Paxton WA, Choe S, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* **1996**;86:367–77.
  6. Dean M, Carrington M, Winkler C, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the *CCR5* structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. *Science* **1996**;273:1856–62.
  7. Samson M, Libert F, Doranz BJ, et al. Resistance to HIV-1 infection in Caucasian individuals bearing mutant alleles of the *CCR-5* chemokine receptor gene. *Nature* **1996**;382:722–5.
  8. Stewart GJ, Ashton LJ, Biti RA, et al. Increased frequency of *CCR-5* delta 32 heterozygotes among long-term non-progressors with HIV-1 infection. The Australian Long-Term Non-Progressor Study Group. *AIDS* **1997**;11:1833–8.
  9. de Roda Husman AM, Koot M, Cornelissen M, et al. Association between *CCR5* genotype and the clinical course of HIV-1 infection. *Ann Intern Med* **1997**;127:882–90.
  10. Ioannidis JP, Rosenberg PS, Goedert JJ, et al. Effects of *CCR5*-delta32, *CCR2*-64I, and *SDF-1* 3'A alleles on HIV-1 disease progression: an international meta-analysis of individual-patient data. *Ann Intern Med* **2001**;135:782–95.
  11. Smith MW, Dean M, Carrington M, et al. Contrasting genetic influence of *CCR2* and *CCR5* variants on HIV-1 infection and disease progression. Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), ALIVE Study. *Science* **1997**;277:959–65.
  12. Kostrikis LG, Huang Y, Moore JP, et al. A chemokine receptor *CCR2* allele delays HIV-1 disease progression and is associated with a *CCR5* promoter mutation. *Nat Med* **1998**;4:350–3.
  13. Yamashita TE, Phair JP, Munoz A, et al. Immunologic and virologic response to highly active antiretroviral therapy in the Multicenter AIDS Cohort Study. *AIDS* **2001**;15:735–46.
  14. Guérin S, Meyer L, Theodorou I, et al. *CCR5* delta32 deletion and response to highly active antiretroviral therapy in HIV-1-infected patients. *AIDS* **2000**;14:2788–90.
  15. Valdez H, Purvis SF, Lederman MM, Fillingame M, Zimmerman PA. Association of the *CCR5Δ32* mutation with improved response to antiretroviral therapy. *JAMA* **1999**;282:734.
  16. O'Brien TR, McDermott DH, Ioannidis JP, et al. Effect of chemokine receptor gene polymorphisms on the response to potent antiretroviral therapy. *AIDS* **2000**;14:821–6.
  17. Brumme ZL, Chan KJ, Dong W, et al. *CCR5Δ32* and promoter polymorphisms are not correlated with initial virological or immunological treatment response. *AIDS* **2001**;15:2259–66.
  18. Bratt G, Karlsson A, Leandersson AC, Albert J, Wahren B, Sandstrom E. Treatment history and baseline viral load, but not viral tropism or *CCR-5* genotype, influence prolonged antiviral efficacy of highly active antiretroviral treatment. *AIDS* **1998**;12:2193–202.
  19. Reijers MH, Weverling GJ, Jurriaans S, et al. Maintenance therapy after quadruple induction therapy in HIV-1 infected individuals: Amsterdam Duration of Antiretroviral Medication (ADAM) study. *Lancet* **1998**;352:185–90.
  20. Reijers MH, Weverling GJ, Jurriaans S, et al. The ADAM study continued: maintenance therapy after 50 weeks of induction therapy. *AIDS* **2001**;15:129–31.
  21. Foudraine NA, Jurriaans S, Weverling GJ, et al. Durable HIV-1 suppression with indinavir after failing lamivudine-containing double nucleoside therapy: a randomized controlled trial. *Antivir Ther* **2001**;6:55–62.
  22. Foudraine NA, de Jong JJ, Jan Weverling G, et al. An open randomized controlled trial of zidovudine plus lamivudine versus stavudine plus lamivudine. *AIDS* **1998**;12:1513–9.
  23. van Griensven GJ, Tielman RA, Goudsmit J, et al. Risk factors and prevalence of HIV antibodies in homosexual men in the Netherlands. *Am J Epidemiol* **1987**;125:1048–57.
  24. Weverling GJ, Lange JM, Jurriaans S, et al. Alternative multidrug regimen provides improved suppression of HIV-1 replication over triple therapy. *AIDS* **1998**;12:F117–22.
  25. van Rij RP, de Roda Husman AM, Brouwer M, Goudsmit J, Coutinho RA, Schuitemaker H. Role of *CCR2* genotype in the clinical course of syncytium-inducing (SI) or non-SI human immunodeficiency virus type 1 infection and in the time to conversion to SI virus variants. *J Infect Dis* **1998**;178:1806–11.
  26. Centers for Disease Control and Prevention. 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Morb Mortal Wkly Rep* **1992**;41(RR-17):1–19.
  27. Lathey JL, Tierney C, Chang SY, et al. Associations of *CCR5*, *CCR2*, and stromal cell-derived factor 1 genotypes with human immunodeficiency virus disease progression in patients receiving nucleoside therapy. *J Infect Dis* **2001**;184:1402–11.
  28. Koot M, van Leeuwen R, de Goede RE, et al. Conversion rate towards a syncytium-inducing (SI) phenotype during different stages of human immunodeficiency virus type 1 infection and prognostic value of SI phenotype for survival after AIDS diagnosis. *J Infect Dis* **1999**;179:254–8.
  29. Michael NL, Chang G, Louie LG, et al. The role of viral phenotype and *CCR-5* gene defects in HIV-1 transmission and disease progression. *Nat Med* **1997**;3:338–40.
  30. Furtado MR, Callaway DS, Phair JP, et al. Persistence of HIV-1 transcription in peripheral-blood mononuclear cells in patients receiving potent antiretroviral therapy. *N Engl J Med* **1999**;340:1614–22.
  31. Imamichi H, Crandall KA, Natarajan V, et al. Human immunodeficiency virus type 1 quasi species that rebound after discontinuation of highly active antiretroviral therapy are similar to the viral quasi species present before initiation of therapy. *J Infect Dis* **2001**;183:36–50.
  32. Kuster H, Opravil M, Ott P, et al. Treatment-induced decline of human immunodeficiency virus-1 p24 and HIV-1 RNA in lymphoid tissue of patients with early human immunodeficiency virus-1 infection. *Am J Pathol* **2000**;156:1973–86.
  33. Fischer M, Gunthard HF, Opravil M, et al. Residual HIV-RNA levels persist for up to 2.5 years in peripheral blood mononuclear cells of patients on potent antiretroviral therapy. *AIDS Res Hum Retroviruses* **2000**;16:1135–40.
  34. Gunthard HF, Frost SD, Leigh-Brown AJ, et al. Evolution of envelope sequences of human immunodeficiency virus type 1 in cellular reservoirs in the setting of potent antiviral therapy. *J Virol* **1999**;73:9404–12.