Dominant Effects of *CCR2–CCR5* Haplotypes in HIV-1 Disease Progression

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Summary: Three haplotypes for the *CCR2–CCR5* region previously have been shown to affect AIDS progression; however, it is not known if the protective and accelerating effects of the haplotypes are relatively constant throughout infection or exert their effects early or late in HIV type 1 infection. The authors report the relative contributions to AIDS progression of *CCR2* 64I, *CCR5* Δ32, and the *CCR5* promoter haplotype +.P1.+ in the GRIV cohort, which included patients representing the extremes of the distribution for AIDS progression: rapid progressors (RP) who developed $CD4^+$ T-cell counts of <300/ mm^3 within 3 years after the last HIV-1–seronegative test and slow progressors (SP) who were HIV-1 infected for ≥8 years with $CD4^+$ T-cell counts of >500/mm^3. Comparing the RP with a seroconverter control group including intermediate progressors to AIDS, we observed the early protective effect of *CCR5* Δ32 (odds ratio = 0.25; $P = 0.007$) was similar in strength to the early susceptible effect of *CCR5* +.P1.+ (odds ratio = 2.1, $P = 0.01$). Comparison of the intermediate control group to the SP showed weaker and less significant odd ratios, suggesting that the effect of these factors tended to be stronger on early progession; the tendency towards a disproportionately early effect was significant for *CCR5* Δ32 ($P = 0.04$) but not for *CCR5* +.P1.+ ($P = 0.12$). Follow-up of SP demonstrated that these polymorphisms have little effect after 8 years, because the subset of SP who had progression after study entry had the same genotype distribution as the global population of SP, suggesting that factors other than *CCR5* or *CCR2* genetic variants must be responsible for the long-term maintenance of nonprogression.

Key Words: AIDS, *CCR2*, *CCR5*, haplotype, HIV, disease progression

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The inhibitory role of chemokines on HIV type 1 (HIV-1) replication was revealed in 1995,[1] and the following year, the receptors *CCR5* and *CXCR4* were identified as the major coreceptors for HIV-1 cell entry.[2–4] Since then, gene polymorphisms in the chemokine system have been investigated intensively in AIDS cohorts to define their role in HIV-1 resistance and pathogenesis.[5] A 32-bp deletion, *CCR5* Δ32, was found in the main chemokine coreceptor for transmitted R5 HIV-1 strains. This deletion introduces a premature stop codon, which results in a truncated protein not expressed on the cell surface and is associated with resistance to HIV-1 infection in homozygotes and a 2- to 4-year delay in developing AIDS in heterozygotes.[6,7] A conservative replacement of valine by isoleucine in a transmembrane domain of the chemokine receptor *CCR2* was also shown to delay time to AIDS by 2–4 years, although the mechanism for this effect is unknown.[8] Finally, the A allele at position 59029G/A, found only on the *CCR5* promoter haplotype P1, was associated with rapid progression to AIDS.[9–11] The 59029A allele has been reported to upregulate transcription in reporter assays[9] and to be associated with higher numbers of *CCR5*-expressing $CD4^+$ cells.[12] These 3 AIDS-affecting alleles occur on 3 haplotypes: 64I.P1.+, +.P1.+, and +.P1.Δ32 for *CCR2*, *CCR5* promoter, and *CCR5* open reading frame, respectively, with + representing the most common allele at each locus. Although other polymorphisms have been identified in the *CCR5* region,[13–15] they are in linkage disequilibrium with the ones mentioned above, are very

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rare, or have little or no effect on HIV-1 pathogenesis. Associations between HIV-1 disease and genetic variants of the ligands for chemokine coreceptors have also been described.[16–19]

The AIDS-modifying effects of *CCR2* 64I, *CCR5* Δ32, and *CCR5* promoter gene polymorphisms on longitudinal seroconverter cohorts have been confirmed,[20,21] but the degree of their influence was quite variable likely due to differences in cohort design and power.[22] The present study analyses *CCR5* and *CCR2* haplotypes in the GRIV cohort,[23–28] bringing into new light the temporal effects of the chemokine receptor gene polymorphisms.

MATERIALS AND METHODS

Subjects

The GRIV cohort was established in 1995 in France to generate a collection of DNA samples for studies of genetic factors that may influence the rate of progression to AIDS.[24] To avoid potential confounding associated with racial/ethnic differences and population substructure in the analysis, only Caucasians of European descent were recruited from hospital-based AIDS units throughout France. Slow progressors (SP) were defined as asymptomatic individuals who had tested HIV-1 seropositive for >8 years with a $CD4^+$ cell count of >500/mm^3 in the absence of antiretroviral therapy. Of the SP, 150 had follow-up for 3 years after enrollment in the GRIV cohort. Rapid progressors (RP) were stringently defined as having a CD4 cell count of <300/mm^3 <3 years after the last seronegative test. Complete genotyping was available for 80 RP and 250 SP.

As an intermediate control group, we used European American seroconverters from the US-based ALIVE[29] and MACS[30] natural history cohorts from whom subjects meeting the criteria for the GRIV RP or SP were excluded. This seroconverter group is reasonable as a control group for 3 reasons: 1) the US-based controls are of Caucasian European ancestry; 2) the allele frequencies of *CCR2* 64I, *CCR5* Δ32, and *CCR5* P1 among the European American population are quite similar to those previously reported for western European cohorts[31,32]; and 3) because the MACS and ALIVE cohorts are true cohorts of seroconverters, they predominantly represent subjects whose diseases progress at average rates. By excluding RP and SP from this group, we obtained an effective control group for the very rapid and slow GRIV groups.

Genetic Typing of *CCR5* Δ32, *CCR2* 64I, and *CCR5* P1

Genotypes for *CCR5* P1–P4, *CCR5* +/Δ32, and *CCR2* V64I were obtained by single-strand conformational polymorphism analysis, polymerase chain restriction–restriction fragment length polymorphism analysis, and 5′-endonuclease (TaqMan) assay as previously described.[8,10,11] Because the *CCR5* promoter haplotype P1, but not P2–P4, was found to modify AIDS in previous studies, we grouped the *CCR5* P2, P3, and P4 haplotypes into a single covariate, P2–P4, in the analysis. The haplotypes considered are thus +.P1.+, 64I. P1.+, +.P1.Δ32, and +.P2–P4.+ for *CCR2*, *CCR5* promoter, and *CCR5* open reading frame, respectively, where + represents the most common allele.[10,11] For brevity, the haplotypes are abbreviated P1, 64I, Δ32, and P2-4, respectively. The P1 haplotype (frequency [f] = 35%) includes the subset of haplotypes HHE (f = 30%), HHF1 (f = 3.0%), and HHG1 (f = 2%) as reported by Gonzalez et al.[33]

Statistical Analysis

Because of the complete linkage disequilibrium within the region with both *CCR2* 64I and *CCR5* Δ32 always occurring with *CCR5* P1 but never together on the same haplotype, we did not consider the independent affects of the *CCR2* and *CCR5* alleles on progression but instead analyzed the effects of haplotypes containing the 3 loci. For each haplotype, we compared each of the groups (RP and SP) with the intermediate control group and RP with SP (Table 1). To test the hypothesis that the protective effects of *CCR5* Δ32 and the accelerating effects of *CCR5* P1 would influence late events in SP, the haplotype distributions between progressing SP and slow SP were compared under the dominant model (see Results and Table 2). Statistical significance was determined by Pearson χ^2 and Fisher exact tests (2 × 2 contingency tables). Confidence intervals for odds ratios (OR) were calculated using PROC FREQ in SAS (SAS Institute, Cary, NC). The hypothesis that the effects of *CCR5* Δ32 and *CCR5* P1 occur disproportionately in early progression was tested by the likelihood ratio statistics comparing the results of the proportionately odds model (function polr) to a multinominal model (function multinom).[34] Because *CCR2* 64I and *CCR5* Δ32 are each in complete linkage disequilibrium with *CCR5* P1, haplotypes were easily determined by inspection.

RESULTS

Table 1 presents the distribution of homozygotes and heterozygotes (dominant model) for haplotypes by comparing the considered haplotype with all other haplotypes. The strongest associations were observed for individuals with either *CCR5* P1 or *CCR5* Δ32. The *CCR5* P1 haplotype was strongly associated with rapid progression, with the highest frequency of P1 carriers observed for RP compared with either SP (OR = 2.96; $P = 0.00006$) or the intermediate control group (OR = 2.10; $P = 0.01$), demonstrating that the *CCR5* P1 haplotype is dominantly associated with rapid progression. In contrast to *CCR5* P1, *CCR5* Δ32 heterozygotes were underrepresented in RP compared with either SP (OR = 0.16; $P = 0.00004$) or the intermediate control group (OR = 0.25; $P = 0.007$). Heterozygotes for the *CCR5* Δ32 haplotype were

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TABLE 1. Frequency Distributions of *CCR2–CCR5* Haplotypes for the Dominant Genetic Model and of *CCR2–CCR5* Diplotypes

	No. (%) RP	No. (%) Intermediate Controls	No. (%) SP	RP vs. Intermediate Controls			Intermediate Controls vs. SP			RP vs. SP		
				OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Haplotype*												
P2–P4	59 (73.8)	156 (75.4)	193 (77.2)	0.92	0.51–1.66	0.76	0.90	0.59–1.39	0.66	0.83	0.46–1.48	0.55
64I	9 (11.3)	40 (19.3)	48 (19.2)	0.53	0.24–1.15	0.12	1.01	0.63–1.61	1	0.53	0.24–1.14	0.13
P1	57 (71.3)	112 (54.1)	114 (45.6)	2.10	1.21–3.67	0.01	1.41	0.97–2.04	0.08	2.96	1.71–5.09	0.00006
Δ32	4 (5)	36 (17.4)	62 (24.8)	0.25	0.09–0.73	0.007	0.64	0.4–1.01	0.07	0.16	0.05–0.45	0.00004
Diplotype												
P2–P4/P2–P4	17 (21.3)	44 (21.3)	55 (22)	1	0.53–1.88	1	0.96	0.61–1.5	0.91	0.95	0.52–1.77	1.000
P2–P4/64I	5 (6.3)	22 (10.6)	30 (12)	0.56	0.21–1.54	0.37	0.87	0.49–1.56	0.66	0.48	0.18–1.30	0.21
P2–P4/P1	36 (45)	69 (33.3)	69 (27.6)	1.64	0.97–2.77	0.08	1.31	0.88–1.96	0.19	2.14	1.28–3.61	0.006
P2–P4/Δ32	1 (1.2)	21 (10.1)	39 (15.6)	0.11	0.02–0.85	0.01	0.61	0.35–1.08	0.10	0.06	0.01–0.51	0.0001
64I/64I	0†	4 (1.9)	5 (2)	0	—	0.58	0.97	—	1	0	—	0.34
64I/P1	4 (5)	10 (4.8)	6 (2.4)	1.04	0.32–3.41	1	2.06	0.74–5.78	0.20	2.14	0.58–7.78	0.26
64I/Δ32	0	4 (1.9)	7 (2.8)	0	—	0.58	0.68	—	0.76	0	—	0.20
P1/P1	14 (17.5)	22 (10.6)	23 (9.2)	1.78	0.86–3.69	0.16	1.17	0.63–2.17	0.64	2.09	1.02–4.29	0.07
P1/Δ32	3 (3.8)	11 (5.3)	16 (6.4)	0.69	0.19–2.56	0.76	0.82	0.37–1.81	0.69	0.57	0.16–2.00	0.58

*Heterozygous individuals were counted twice, while homozygous individuals were counted once.
†No CIs calculated for OR = 0 (no failure in the group).
CI indicates confidence interval.

slightly more frequent, and heterozygotes for *CCR5 P*1 slightly less frequent, in SP than in the intermediate control group, although neither tendency was significant. These results suggested that both *CCR5* Δ32 and *CCR5* P1 haplotypes exert their effects very early, because the largest distortion in haplotype frequencies was noted for RP compared with the intermediate control group with smaller differences observed between SP and the intermediate control group. We tested this hypothesis by comparing the results of a regression model (proportional odds) that assumes that effects are constant, to the results of a regression model (multinomial) that allows the effects to vary with time; the comparison indicated that the effect of *CCR5* Δ32 differed significantly from a constant effect ($P = 0.04$), while the effect of *CCR5* +/P1/+ did not differ significantly ($P = 0.12$).

To determine the effects of haplotype pairs (diplotypes) on progression, we examined the distribution of diplotypes among SP, RP, and the intermediate control group (Table 1). The P2-4/P2-4 diplotype was neutral, because the frequency was nearly identical among the 3 groups (f = 21%–22%). Individuals carrying 1 *CCR5* Δ32 haplotype, regardless of the second haplotype, tended to be underrepresented in RP and overrepresented in SP. By comparing RP with the intermediate control group, we observed that the *CCR5* P2-4/Δ32 diplotype is strongly protective (OR = 0.11; $P = 0.01$).

Of SP, 150 had follow-up clinical visits over 3 years: 45 remained slow SP with no treatment and stable $CD4^+$ cell counts (<20% decline in $CD4^+$ T-cell count); 47 progressing SP had sharp declines in $CD4^+$ cell counts to <400/mm^3 and/or received antiretroviral therapy; and 58 had a slow decrease in $CD4^+$ cell counts that remained >500/mm^3. There were no significant differences among progressing SP, slow SP, and the remaining 58 SP ($P > 0.3$; Table 2), confirming that the AIDS-modifying genetic factors that influence viral cell entry exert their effects early in HIV-1 infection.

TABLE 2. Distribution of *CCR2–CCR5* Haplotype Among Patients With 3 Years of Clinical Follow-up After Enrollment in the GRIV Cohort According to the Dominant Model

Haplotype*	No. (%) Slow SP† n = 45	No. (%) Other SP‡ n = 250	No. (%) Progressing SP§ n = 47
P2–P4	34 (75.5)	43 (74.1)	35 (74.5)
64I	6 (13.3)	12 (20.7)	10 (21.3)
P1	21 (46.7)	26 (44.8)	21 (44.7)
Δ32	11 (24.4)	11 (19.0)	13 (27.7)

All comparisons 2 × 2 contingency tables led to $P > 0.3$.
*Heterozygotes were counted twice, and homozygotes were counted once.
†Slow SP who did not have $CD4^+$ cell counts progress to <500/mm^3 or received highly active antiretroviral therapy.
‡Includes nonclassified SP.
§Progressing SP with a decline in $CD4^+$ cell count to <400/mm^3 or who received highly active antiretroviral therapy during the 3-year interval.

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DISCUSSION

In this study, comparison of subjects progressing in the first three years after HIV-1 infection with average progressors showed that *CCR5* Δ32 and *CCR5* P1 have a strong influence early in HIV-1 infection. The effect of these factors tended to be stronger on early progression than on late progression, although this tendency was significant only for *CCR5* Δ32. It is likely that initial viral load is diminished by the presence of *CCR5* Δ32 and increased by *CCR5* P1[20,21]; however, we could not access this because early set point RNA levels were not available for the GRIV cohort. Carrying *CCR5* Δ32 and not *CCR5* P1 might provide an initial advantage in limiting $CD4^+$ cell depletion early in infection. As a consequence, patients with *CCR5* Δ32 would be more likely to be SP according to our criteria ($CD4^+$ cell counts of >500/mm^3 for at least the first 8 years after HIV-1 seroconversion) as shown by the increase of patients with *CCR5* Δ32 and the decrease of those with *CCR5* P1 among SP compared with the intermediate control group (Table 1).

These studies demonstrate that while both *CCR5* Δ32 and *CCR5* P1 are dominant, the protective effect of *CCR5* Δ32 may be more influential because patients with the *CCR5* Δ32/P1 diplotype were 60% more likely to be SP or in the intermediate control group than RP; however, these are to be considered as trends due to the small number of individuals carrying the *CCR5* Δ32/ P1 diplotype (Table 1). *CCR5* P1 was found in some[10,19] but not other[9,11] studies to be recessive. The current study provides convincing evidence that *CCR5* P1 is dominant but only when the trans (second) haplotype carried by an individual is *CCR5* P2, P3, or P4. We were unable to assess the dominance of *CCR5* P1 in individuals with either *CCR5* Δ32 or *CCR2* 64I because the groups were underpowered; however, there appeared to be a tendency for *CCR5* Δ32, but not *CCR2* 64I, to mitigate the accelerating effects of *CCR5* P1 in trans. The epidemiologic evidence that *CCR5* P1 acts early to increase the risk of AIDS progression and $CD4^+$ T-cell loss is consistent with evidence that *CCR5* P1 has been associated with higher transcriptional levels[9] and increased numbers of *CCR5*-expressing $CD4^+$ cells (12αα), thus providing more targets for HIV-1 binding. Studies have consistently shown that set point HIV-1 levels are predictors of AIDS progression[35,36]; therefore genetic factors that modify chemokine receptor availability may have long-term effects on HIV-1 pathogenesis. The early detrimental effects of *CCR5* P1 suggest that HIV-1–infected carriers of this factor could be considered for early therapeutic surveillance.

We did not observe a significant association of the *CCR2* 64I haplotype on slow or rapid progression (Table 1), unlike observations in some other studies[20]; however, the comparison of SP with the intermediate control group or RP suggests that in the absence of *CCR5* +/P1/+, *CCR2* 64I tends to be protective. Globally, the pattern of protection brought by *CCR2* 64I seems complex, with a trend for an early protective effect against rapid progression (Table 1) and a trend for delayed protection shown by an increased frequency among SP but modulated by *CCR5* P1 (Table 1). This observation is in agreement with the findings of Ioannidis et al[37] who studied perinatally infected children. The antagonistic effect of the *CCR5* P1 haplotype on the *CCR2* 64I haplotype in trans (Table 1) suggests that upregulation of *CCR5* by *CCR5* P1 may be more important than the presence or absence of *CCR2* 64I. The functional role of *CCR2* 64I remains an enigma both because the valine to isoleucine substitution is conservative and occurs within a transmembrane domain and *CCR2* is a minor HIV-1 coreceptor with little evidence that it binds to HIV-1 in vivo. It is possible that *CCR2* 64I is tracking other yet unidentified polymorphisms in linkage disequilibrium with *CCR5* or other nearby chromosome 3 chemokine receptors.[8,11]

Follow-up data were available for a subset of SP. As shown in Table 2, among SP with signs of progression or remaining stable after 3 years, the *CCR5* Δ32 and *CCR2* 64I haplotype frequencies were quite similar; this suggests that these genetic factors do not provide long-term protection, in agreement with results of previous studies.[8,9,24,25] Similarly, the frequencies of *CCR5* P1 and *CCR5* P2-4 haplotypes were also similar among SP regardless of their progression status. These results support our observation that *CCR2–CCR5* genetic factors function early and may have little influence on later events triggering $CD4^+$ T-cell loss in individuals whose conditions have been stable for ≥8 years, in agreement with results of previous studies.[37,38] This suggests that other genetic, viral, or environmental factors may be responsible for the long-term maintenance of nonprogression.

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