Genetic Acceleration of AIDS Progression by a Promoter Variant of *CCR5*

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The *CCR5* gene encodes a cell surface chemokine receptor molecule that serves as the principal coreceptor, with CD4, for macrophage-tropic (R5) strains of human immunodeficiency virus–type 1 (HIV-1). Genetic association analysis of five cohorts of people with acquired immunodeficiency syndrome (AIDS) revealed that infected individuals homozygous for a multisite haplotype of the *CCR5* regulatory region containing the promoter allele, *CCR5P1*, progress to AIDS more rapidly than those with other *CCR5* promoter genotypes, particularly in the early years after infection. Composite genetic epidemiologic analyses of genotypes bearing *CCR5P1*, *CCR5-Δ32*, *CCR2-64I*, and *SDF1-3′A* affirmed distinct regulatory influences for each gene on AIDS progression. An estimated 10 to 17 percent of patients who develop AIDS within 3.5 years of HIV-1 infection do so because they are homozygous for *CCR5P1/P1*, and 7 to 13 percent of all people carry this susceptible genotype. The cumulative and interactive influence of these AIDS restriction genes illustrates the multigenic nature of host factors limiting AIDS disease progression.

The human *CCR5* and *CCR2* chemokine receptor genes, which serve as coreceptors with CD4 for HIV, are tightly linked on chromosome 3p21-22, separated by 20 kb (*1–4*). Common allelic variants in both genes are associated with slower progression to AIDS after infection (*1, 5–11*). The protective influences of *CCR5-Δ32* and *CCR2-64I* are independent in AIDS cohorts, and the two mutations have never been found on the same chromosome haplotype (*5, 10*). The physical proximity of *CCR2* and *CCR5*, the equivalent functional efficiency of alternative *CCR2* allelic products as chemokine or HIV-1 coreceptors (*9*), and the rarity of HIV-1 strains that use the CCR2 receptor (*4*) have led to the speculation that *CCR2-64I* may be hitchhiking (or tracking by linkage disequilibrium) with an undiscovered *CCR5* variant, perhaps in the cis-regulatory region, that is directly responsible for the *CCR2-64I* protective effect (*5, 9–11*).

The promoter region of *CCR5* has been characterized, and transcription is regulated by two domains, the stronger of which is located within the region that includes intron 1, exon 2, and part of exon 3 (*12–14*). Four common allelic variants (*CCR5P1–P4*) were detected in a denaturing high-pressure liquid chromatography (DHPLC) screen of AIDS patients (Fig. 1) (*15*). Six rare alleles (*CCR5P5–P10*) were discovered as heterozygotes upon subsequent single-strand conformation polymorphism (SSCP) screening of five AIDS cohorts (*15*). Sequence analysis of the *CCR5* promoter region of individuals homozygous for the *CCR5P1–P4* variants and heterozygotes of the six rare variants revealed 10 polymorphic nucleotide positions that describe the 10 *CCR5* promoter haplotype alleles, referred to as promoter alleles (Fig. 1).

An SSCP-based genotype survey of *CCR5P* alleles among 2603 individuals enrolled in five AIDS cohorts (*1, 5, 16, 17*) indicated that *CCR2-64I* was always found on a *CCR5P1*-bearing haplotype and that *CCR5-Δ32* was consistently found on a *CCR5P1* haplotype as well. This conclusion is derived from the observation that *CCR2-64I/64I* homozygotes were always *CCR5P1/P1* homozygotes ($N = 43$) and *CCR2-+/64I* heterozygotes ($N = 559$) were invariably heterozygous or homozygous for *CCR5P1*. Similarly, *CCR5-Δ32/Δ32* homozygotes were always *CCR5P1/P1* homozygotes ($N = 18$), whereas *CCR5-+/Δ32* heterozygotes were invariably heterozygous or homozygous for *CCR5P1* ($N = 298$). Finally, none of 657 individuals who lacked the *CCR5P1* allele had either the *CCR5-Δ32* or the *CCR2-64I* allele ($P < 0.0001$). Because of these associations, the entire *CCR2-CCR5* complex can be considered as a six-allele genotype system, based on the composite [*CCR2(+/64I).CCR5P(P1–P4).CCR5(+/Δ32)*] haplotype (*CCR5P* denotes promoter region alleles; *CCR5* refers to the coding region alleles). The haplotypes using the same locus order are [*+.P1.+*], [*64I.P1.+*], [*+.P1.Δ32*], [*+.P2.+*], [*+.P3.+*], and [*+.P4.+*]. The six respective haplotype frequencies observed in Caucasians ($N = 1383$) were 0.358, 0.098, 0.104, 0.085, 0.14, and 0.354; in African Americans ($N = 1006$) the respective frequencies were 0.258, 0.155, 0.018, 0.229, 0.191, and 0.147 (*18*).

The frequency distribution of *CCR5* promoter alleles and alternative genotypes was compared among 474 uninfected individuals from "at risk" cohorts and 1353 HIV-1–infected patients stratified by ethnic group. No significant differences in *CCR5P* allele or genotype frequencies were observed in Caucasians or African Americans or in a more stringent analysis of documented high-risk uninfected individuals from the MACS and MHCS cohorts (*17, 18*).

The influence of *CCR5* promoter alleles on the rate of AIDS progression among HIV-1–infected Caucasian ($N = 700$) and African American ($N = 162$) seroconverters (individuals whose infection date can be estimated as the time between the last negative HIV-1 antibody test and the first positive test) was examined in a survival analysis. A Cox proportional hazards model (*19, 20*) was used to compare progression to AIDS among genotype combinations of common *CCR5P* alleles for combined and separate cohorts, including those *CCR5*, *CCR2*, and *SDF1* genotypes previously shown to confer resistance to AIDS progression (*20, 21*). Four AIDS endpoints reflecting advancing morbidity were evaluated: (i) CD4 < 200 cells/mm^2; (ii) AIDS-1993, as defined by the U.S. Centers for Disease Control and Prevention (*22*) (that is, HIV-1 infection plus AIDS-defining illness or decline of CD4 T lymphocytes to <200 cells/mm^3), or death; (iii) the more stringent AIDS-1987 definition (*22*) (HIV-1 infection plus AIDS-defining illness), or death; and (iv) death during follow-up for an HIV-1–infected patient.

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Aside from the previously demonstrated *CCR2-64I*–, *CCR5-Δ32*–, and *SDF1-3′A/3′A*–mediated delay in AIDS, the only consistent genotypic association was an accelerated AIDS progression among individuals homozygous for the *CCR5P1/P1* genotype. This rapid progression was highly significant for AIDS-1987 among combined Caucasian cohorts [N = 700; relative hazard (RH) = 1.53, P = 0.005], and was significant for both homosexual (MACS) and hemophiliac (MHCS) cohorts for AIDS-1993 (P = 0.06 and 0.003, respectively) and AIDS-1987 endpoints (P = 0.03 and 0.002, respectively) (*18*).

To assess the role of *CCR5P1* in the context of *CCR2-64I* and *CCR5-Δ32*, we performed a survival analysis considering composite [*CCR2.CCR5P.CCR5*] genotypes (Fig. 2, A and B). The survival analysis for combined Caucasian cohorts shows an accelerated rate to AIDS among [+.*P1.*+]/[+.*P1.*+] homozygotes for each endpoint, relative to patients with other genotypes (RH = 1.5, P = 0.002 to 0.005). The curves reflecting AIDS protection due to *CCR5-+/Δ32*, *CCR2-+/64I*, or *SDF1-3′A/3′A* reaffirm the protective effects of these alleles. Survival curves representing the other genotypes (individuals with *CCR5P2*, *P3*, or *P4*, who typed negative for *CCR5-Δ32*, *CCR2-64I*, or *SDF1-3′A/3′A*) progressed at an intermediate rate between the [+.*P1.*+]/[+.*P1.*+] homozygotes and the *CCR2*, *CCR5*, or *SDF1* protective genotypes. Relative to unadjusted RH, the RH for *CCR5P1/P1* adjusted for the protective effects of *CCR5-Δ32*, *CCR2-64I*, and *SDF1-3′A/3′A* is diminished (Table 1 and Fig. 2, A and B), reflecting the mix of protective and nonprotective genotypes that contribute to the nonaccelerating (referent) group compared with the *CCR5P1/P1* homozygotes alone.

The pattern of [+.*P1.*+]/[+.*P1.*+] acceleration observed in Fig. 2, A and B, suggested that the strength of the promoter genotype effect changed over time after infection, as has been observed previously for *SDF1-3′A/3′A* (*16*). To test this hypothesis, we examined the *CCR5P1/P1* protection with the use of an RH model partitioned over 2-year intervals (*23*). This analysis indicated that RHs for this genotype were statistically significant for intervals less than 4 years with CD4 < 200, AIDS-1987, and AIDS-1993 endpoints, and for intervals less than 6 years with death as an outcome. After this point, the *CCR5P1/P1* influence was no longer evident.

Table 1 summarizes this time dependence by comparing the entire period of follow-up (up to 19 years) with a separate analysis for the *CCR5P1/P1* effect early (<5 years after HIV-1 infection) and late (>5 years after HIV-1 infection). These analyses yielded two separate RH values (for combined cohorts with four AIDS outcomes): (i) adjusted RH (RH_{adj}), where the effects of *CCR2-64I*, *CCR5-Δ32*, and *SDF1-3′A/3′A* are considered as covariables; and (ii) unadjusted RH (RH_u), where individuals with protective genotypes are included but protection is not treated as a covariable. The results affirm the marked influence of the [+.*P1.*+]/[+.*P1.*+] genotype in promoting rapid progression to AIDS, as well as the time dependence of the association. The statistical association is strongest in the early stages of HIV infection and is reduced or absent after 5 years for the three early AIDS endpoints and after 6 years for death (that is, although RH > 1.0, the P values for late stages are all >0.05). The overall and time-dependent effect of *CCR5P1/P1* is apparent in Caucasian homosexual and hemophiliac cohorts analyzed separately as well as when multiple cohorts are combined (Table 2). Further, the nonpromoter protection (that conferred by the *CCR5-Δ32*, *CCR2-64I*, and *SDF1-3′A/3′A* genotypes) explains part, but not all, of the *CCR5P1/P1* effect, as the adjusted RHs are only 10 to 17% lower than the unadjusted RH and remain highly significant (RH ≫ 1.0, $P \leq 0.01$; Table 1) even when the effects of protective genotypes are removed. The African American cohort fails to show the [+.*P1.*+]/[+.*P1.*+] association with rapid progression for any AIDS outcome (N = 160, P = 0.17 to 1.0), and when added to the Caucasians, the RHs for combined ethnic groups decrease compared with those for Caucasians (*18*).

The antagonistic effects of the recessive [+.*P1.*+]/[+.*P1.*+] genotype to accelerate AIDS progression and the dominant [*64I.P1.*+] and [+.*P1.Δ32*] heterozygous genotypes to delay AIDS progression (*1, 5–11*) were examined further in a categorical comparison of genotype frequency distribution across time intervals of AIDS outcomes (*1, 5, 16*) (Fig. 2, C to F). This approach, termed a defined disease category analysis, allows the inclusion of seroprevalent patients (those who enter cohort studies already HIV-1 antibody–positive) in the slow/nonprogressor category. In the absence of *CCR2-64I* or *CCR5-Δ32* genotypic protection, the susceptible genotype [+.*P1.*+]/[+.*P1.*+] shows a significant drop in frequency over increasing periods of HIV-1 infection (P = 0.001 to 0.003; Fig. 2, C and D). Alternatively, individuals heterozygous for *CCR5-+/Δ32* or *CCR2-+/64I* protective genotypes show a significant rise in incidence among patients who avoid AIDS for longer periods (P = 0.0003 to 0.002; Fig. 2, E and F). As the two protective alleles are dominant and are invariably both carried on alternative *CCR5P1* haplotypes ([*64I.P1.*+] and [+.*P1.Δ32*]), an increase in protective heterozygote frequency in slow/nonprogressors suggests that dominant protective alleles are stronger in effect than the cis-associated *CCR5P1* promoter allele, at least as heterozygotes. When homozygotes for *CCR5-P1/P1* occur in individuals that are also heterozygous for protection ([+.*P1.*+]/[*64I.P1.*+] or [+.*P1.*+]/[+.*P1.Δ32*]), their effects are offset by each other, particularly in later years (7 to 20 years after seroconversion), because the frequencies of the combined *CCR5P1/P1* susceptible and *CCR2/CCR5* protective genotypes are not significantly different in patients who survive AIDS for longer periods (P = 0.15 and 0.20 for AIDS-1993 and AIDS-1987, respectively) (*18*). The epidemiologic canceling of *CCR5P1/P1* AIDS acceleration by *CCR5/2* genotype protection implies that the two effects are roughly equivalent (albeit op-

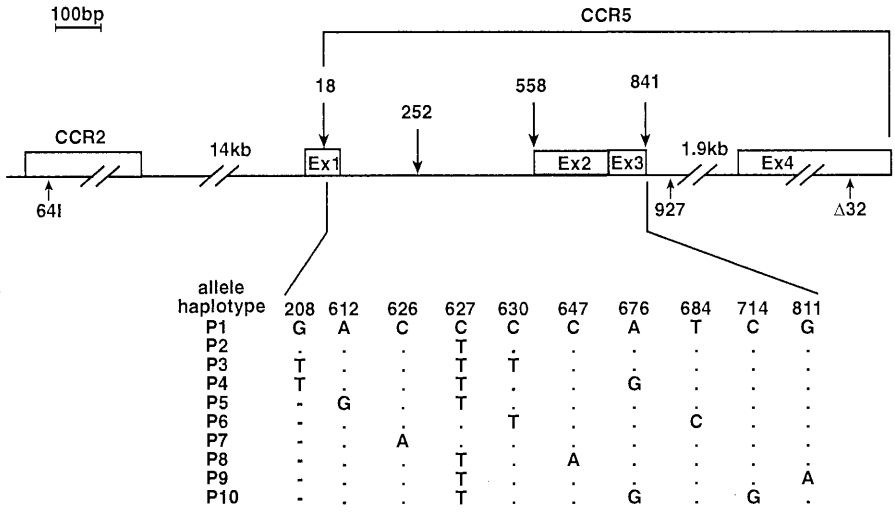

Fig. 1. Map of *CCR2*, *CCR5*, *CCR5* promoter region, and nucleotide variants. SSCP and DHPLC screens (*15*) of two segments of the *CCR5* promoter region (*12*) (positions 18 to 252 and 558 to 841) revealed 10 nucleotide positions with genetic variation. Combinations of the 10 polymorphic nucleotide residues specify 10 *CCR5* promoter region haplotype alleles designated *CCR5P1* through *CCR5P10*. Dots indicate nucleotides identical to those of *CCR5P1*; dashes represent sites in rare haplotypes that were not determined. The mutants *CCR5-Δ32* and *CCR2-64I* are invariably associated in linkage disequilibrium with *CCR5P1*. Sites 208, 627, 676, and 684 were also described in (*12*).

posite in direction) to promote or impede AIDS pathogenesis, respectively.

Kostrikis *et al.* (*10*) recently reported a variant at position 927 (Fig. 1) within intron 2 of the *CCR5* gene, which was in linkage disequilibrium with the AIDS protective variant *CCR2-64I*. Their suggestion that *CCR2-64I*–associated protection might be explained by the *CCR5P-927* variant was examined in the present cohort by typing 984 individuals for *CCR2* and *CCR5P-927* variant (T substituted for C at position 927). All *CCR2-64I*–bearing haplotypes [*64I.P1.+*] contained the *CCR5P-927T* type, confirming the strong linkage disequilibrium association of the two sites. The *CCR5P-927T* variant was also found infrequently on a [*+.P1.+*] haplotype (16/230; 7%). Nine of these individuals were seroconverters, too few for a robust Cox analysis. However, a preliminary analysis of these individuals hinted toward AIDS protection, although none were significant [for CD4 < 200, RH_u = 0.49 (P = 0.31), RH_{adj} = 0.41 (P = 0.21); for AIDS-1993, RH_u = 0.57 (P = 0.34), RH_{adj} = 0.49 (P = 0.22); for AIDS-1987, RH_u = 0.82 (P = 0.78), RH_{adj} = 0.70 (P = 0.61); for death, RH_u = 1.35 (P = 0.67), RH_{adj} = 1.14 (P = 0.86)]. In contrast, an independent study of *CCR2-64I* protection (*24*) showed that a few patients carrying the *CCR5P-927T* variant (*CCR2-+/+*) progressed to AIDS faster than did patients who had the common 927C allele (*18*). The data affirm the association of *CCR2-64I* and *CCR5P-927T*, but neither support nor refute the idea that *CCR5P-927T* adequately explains *CCR2-64I* protection. The opposite and offsetting effects of *CCR5P1* and *CCR2 64I* alleles (respectively to accelerate and delay AIDS; Fig. 2) would imply that their influences involve different mechanisms.

The recessive AIDS accelerating phenotype of the [*+.P1.+*]/[*+.P1.+*] genotype is powerful, apparent in multiple and combined cohorts, and equivalent in strength to protection from AIDS progression afforded by *CCR5-Δ32* or *CCR2-64I* heterozygosity (Fig. 1) (*1, 5–8*). That the acceleration is strongest in the initial 4 to 6 years after infection is consistent with the knowledge that CCR5 is the primary HIV-1 receptor in the early years of infection (*25*). Potential mechanisms of action would include differential constitutive expression of *CCR5* products regulated by various promoter alleles, or, alternatively, differential allele sensitivity to *CCR5* promoter binding proteins, which would regulate transcription (*12, 13, 26, 27*). Quantitative analysis of CCR5 on peripheral blood mononuclear cells (PBMCs) of genotypically *CCR5P1/P1*, *P1/P4*, and *P4/P4* healthy volunteers studied in two separate laboratories (N.L.M. and R.W.D.) revealed a wide range of *CCR5* expression within each genotype but

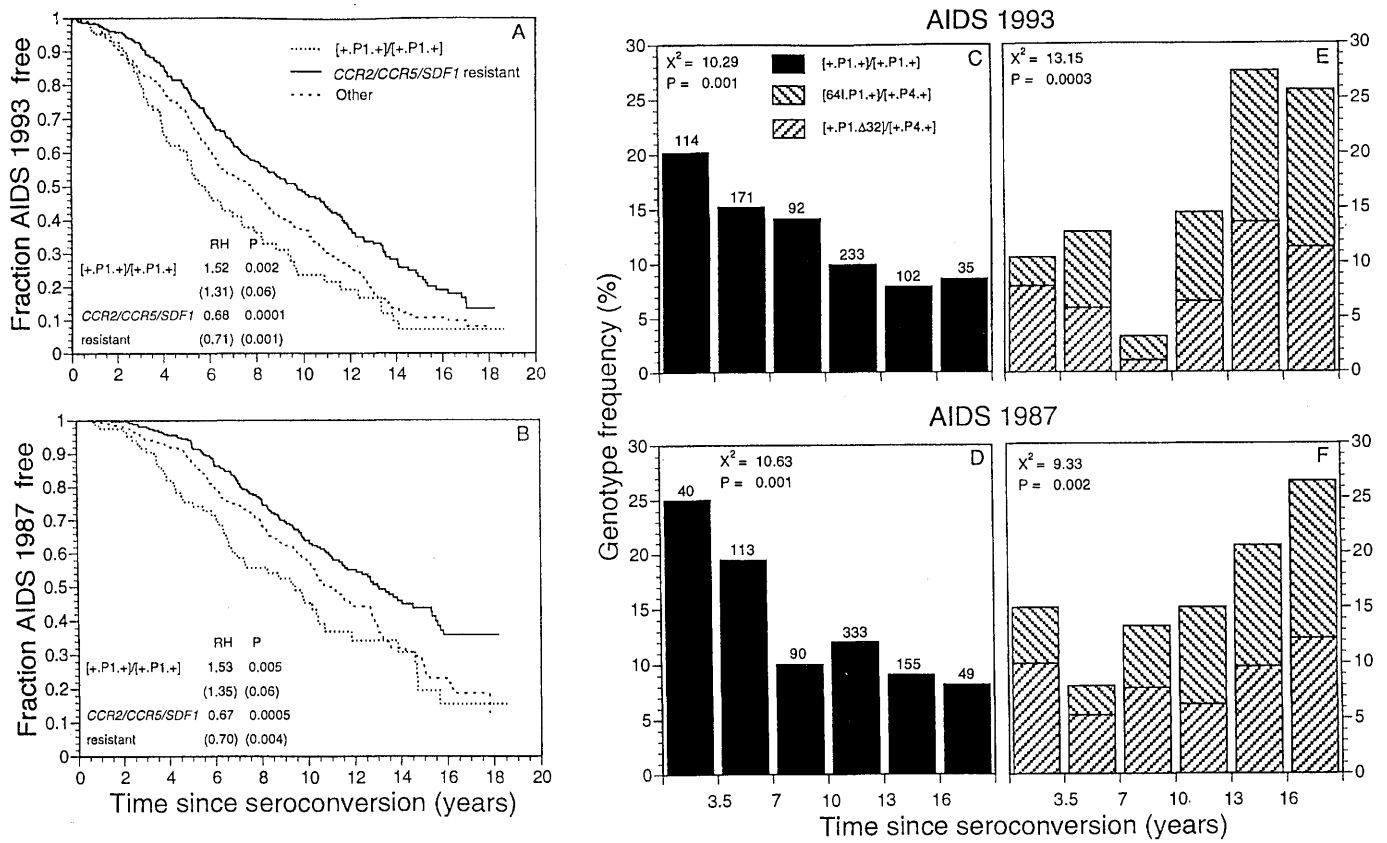

Fig. 2 (A and B). Kaplan-Meier survival curves of seroconverters. Time to AIDS-1993 (A) and AIDS-1987 (B) was examined in Caucasians from all the cohorts. The dotted lines are survival curves for individuals with the [*CCR2-CCR5P-CCR5*]: [*+.P1.+*]/[*+.P1.+*] genotype. The combined protective effects observed in individuals carrying the [*+.P1.Δ32*] and [*64I.P1.+*] haplotypes (or both), with or without the *SDF1-3′A/3′A* protective genotype, are shown by solid lines. Survival curves representing the other genotypes (individuals with *CCR5P2*, *P3*, or *P4*, who typed negative for *CCR5-Δ32*, *CCR2-64I*, or *SDF1-3′A/3′A*) are shown by dashed lines. RH values for genotypic protection by [*+.P1.+*]/[*+.P1.+*] versus all other genotypes and *CCR5-+/Δ32*, *CCR2-+/64I*, or *SDF1-3′A/3′A* (or any combination of these) versus all other genotypes are also shown. The RHs and statistical significance for [*+.P1.+*]/[*+.P1.+*] rapid progression to AIDS, computed by Cox regression analysis, become diminished when they are adjusted to consider the protective influence of *CCR5-+/Δ32*, *CCR2-/64I*, and *SDF1-3′A/3′A* genotypes (in parentheses; see text). (**C** and **D**) Frequencies of the susceptible [*+.P1.+*]/[*+.P1.+*] genotype, in six time classes of progression to AIDS outcomes of AIDS-1993 (C) and AIDS-1987 (D). The genotypic frequencies in each time category reflect only seroconverters before 10 years; after 10 years, time to failure or in study includes both seroprevalents and seroconverters (*5, 16*). (**E** and **F**) Frequencies of the *CCR2* and *CCR5* protective genotypes [*64I.P1.+*]/[*+.P4.+*], [*+.P1.Δ32*]/[*+.P4.+*] in the same six time classes of AIDS progression for AIDS-1993 (E) and AIDS-1987 (F). χ^2 and P values reflect a Mantel-Haenszel test for a linear association between genotypic frequencies and increased time to AIDS-1993 and AIDS-1987 (*36*).

near-equivalent mean concentrations of CCR5, efficiency of promoting luciferase reporter gene, and infectivity by HIV-1 R5-tropic or R5/x4-tropic strains (*28*). These preliminary results would indicate that quantification of CCR5, ligand signaling, and HIV-1–tropic type usage on different cell subsets susceptible to HIV-1 infection (*29*), plus identification of requisite *CCR5* promoter binding factors, would be required to more fully account for *CCR5 P1/P1* action in vivo.

To date, three distinct genetic sites within a short distance on chromosome 3p21 (*CCR2*, *CCR5P*, and *CCR5*; Fig. 1), plus *SDF1* on chromosome 10q11, have been identified as encoding common alleles that independently delimit or increase the rate of AIDS progression among HIV-1–infected carriers (*1, 5–11, 16*). The frequency of individuals who have one or more protective genotypes (*CCR5*, *CCR2*, or *SDF1*) is substantial: 39.1% of Caucasians and 31.5% of African Americans. *CCR5P1* represents the first allelic variant to accelerate AIDS progression, and 12.7% of Caucasians and 6.7% of African Americans carry a *CCR5P1/P1* genotype. Using data from Fig. 2, C and D, we estimated the protected fraction of the *CCR5P1/P1* genotype for rapid (≤3.5 years) progression to AIDS as 10 to 17%, indicating that 10 to 17% of very rapid progressors are in that category because they are homozygous for *CCR5P1/P1* (*30*).

The powerful genetic influence for four chemokine/chemokine receptor loci (*CCR5*, *CCR5P1*, *CCR2*, and *SDF1*) plus the marked effects of *HLA* zygosity on AIDS progression (*1, 5–11, 16, 31–33*), combined with demonstrated functions for their gene products in AIDS pathogenesis (*25*), provide a compelling example of multigenic influence on HIV-1 disease progression. These findings may provide a basis for the development of therapeutic applications as well as for the resolution of other complex polygenic human diseases, including those that require environmental contingencies (such as viral exposure) for phenotype recognition.

Note added in proof: After this report was submitted, McDermott *et al.* (*34*) reported a G/T variant, corresponding to position 303 of the promoter region (Fig. 1), that showed an epidemiological association with rapid progression to AIDS. Alleles at this site were not assessed in the present report but may track the same effect due to linkage disequilibrium with *CCR5P1*.

Table 1. Survival analyses of progression to AIDS outcomes by Caucasians with the *CCR5P1/P1* genotype, with (RH_{adj}) and without (RH_u) adjustment for *CCR5*, *CCR2*, and *SDF1* protective genotypes.

AIDS endpoint	Time interval (years)	*n*/events	RH_u	*P*	RH_{adj}	*P*
CD4 < 200	0–19	694/399	1.55	0.003	1.31	0.08
	0–5	694/136	1.83	0.005	1.52	0.07
	>5	694/263	1.36	0.13	1.18	0.43
AIDS-1993	0–19	694/461	1.52	0.002	1.34	0.04
	0–5	694/178	1.76	0.003	1.52	0.04
	>5	694/283	1.33	0.14	1.19	0.38
AIDS-1987	0–19	700/340	1.53	0.005	1.40	0.04
	0–5	700/77	2.50	0.0004	2.22	0.003
	>5	700/263	1.23	0.27	1.14	0.51
Death	0–19	700/263	1.41	0.05	1.24	0.25
	0–6	700/60	2.07	0.02	1.89	0.04
	>6	700/203	1.19	0.44	1.02	0.92

Table 2. Analysis of protection against AIDS outcomes by the *CCR5-P1/P1* genotype in separate homosexual and hemophiliac cohorts.

AIDS endpoint	Cohort	0 to 19 years			0 to 5 years*		>5 years	
		n/events	RH_u	*P*	RH_u	*P*	RH_u	*P*
CD4 < 200	CAUC	694/399	1.55	0.003	1.83	0.005	1.36	0.13
	MACS	365/190	1.42	0.09	1.34	0.25	1.59	0.18
	MHCS	164/106	2.55	0.002	6.69	0.0002	1.76	0.14
AIDS-1993	CAUC	694/461	1.52	0.002	1.76	0.003	1.33	0.14
	MACS	365/293	1.42	0.06	1.32	0.21	1.68	0.11
	MHCS	164/112	2.40	0.003	6.80	0.0001	1.47	0.34
AIDS-1987	CAUC	700/340	1.53	0.005	2.50	0.0004	1.23	0.27
	MACS	370/191	1.51	0.03	2.25	0.005	1.14	0.62
	MHCS	164/78	2.66	0.002	6.91	0.002	2.03	0.07
Death*	CAUC	700/263	1.41	0.05	2.07	0.02	1.19	0.44
	MACS	370/151	1.38	0.15	1.64	0.15	1.23	0.50
	MHCS	164/62	2.64	0.009	4.87	0.03	1.66	0.21

*For death a cutoff of 6 years was used.

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15. The region between exons 1 and 3 of the 5′ untranslated region of the *CCR5* gene was analyzed by amplification of overlapping segments of 200- to 300-bp DNA fragments using the following primer pairs: F9 (5′-GATTCTGTGTAGTGGGATGAGC) and R9 (5′-GAGTTTCTTGTAGGGGAACGG), positions 558 to 841; F10 (5′-TAGCCTTACTGTTGAAAAGCCC) and R10 (5′-GTTTTTGTTCTCTGCTCATCCC), positions 410 to 592; F11 (5′-ATATTGGGTGGTGAGCATCTG) and R11 (5′-ATTCATCTAGTCAAAAGCCCAC), positions 231 to 507; F12 (5′-CTGGAGTGAAGAATCCTGCC) and R12 (5′-ACAGATGCTCACCACCCAAT), positions 18 to 252 [numbering system based on (*12*)]. Polymerase chain reactions were performed using a modified step program [K. H. Hecker and K. K. Roux, *Biotechniques* **20**, 478 (1986)] in which the annealing temperature was 65°C for one cycle and lowered 0.5°C every cycle to 59°C, for 30 s each cycle. Thirty additional cycles were then performed at 50°C annealing temperature. DHPLC was performed as described [P. A. Underhill *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 195 (1996)]. Products were run on a Varian/Rainin machine using a DNASep column (Transgenomic), and the DNA was detected by ultraviolet fluorescence at 254 nm. Samples were run in 0.1 M triethylamine acetate buffer, pH 7 (Perkin-Elmer) containing acetonitrile (Baker). Samples were injected at 17.5% acetonitrile, raised to 30.5% at 1 min, to 34% over the next 4 min, and to 50% for the last 2 min. SSCP methods are as described by M. Cullen *et al.* [*Am. J. Hum. Genet.* **60**, 397 (1997)].
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20. Seroconverter individuals in each of three cohorts (MACS, MHCS, ALIVE) plus combined cohorts (ALL) were analyzed by means of Cox proportional hazards model (PROC PHREG, SAS Institute, Cary, NC) (*19*) for all analytical combinations of genotype, AIDS outcome, cohort, and ethnic group by stepwise regression. Homozygotes (recessive model), heterozygotes (codominant model), and sum of the homozygotes plus heterozygotes (dominant model) for each of six haplotype-bearing genotypes were considered for three polymorphic loci (*CCR2*, *CCR5P*, and *CCR5*). The six allelic haplotypes were [+.*P1*.+], [+.*P1*.Δ*32*], [*64I*.*P1*.+], [+.*P2*.+], [+.*P3*.+], and [+.*P4*.+]. These were common haplotypes for the three loci in Cau-

casian and African Americans. The six haplotypes occur in 21 different genotypes that were tested versus other haplotype genotype combinations for progression rate to AIDS using the CDC case definition of AIDS from 1993 (*22*). We also tested separate and combined cohorts for the various genotypes effect on AIDS progression using the more stringent 1987 AIDS definition (*22*). In African Americans, all of the promoter allele genotypes were considered in Cox analyses, of which none were significant. Bonferroni corrections for multiple tests were performed as described [B. S. Weir, *Genetic Data Analysis* (Sinauer, Sunderland, MA, 1990); T. Schweder and E. Spjotvoll, *Biometrika* **69**, 493 (1982)].

21. The AIDS protective effects of the three genotypes *CCR5-+/Δ32*, *CCR2-+/64I*, and *SDF1-3′A/3′A* observed in the same cohorts (*1, 5–11, 16*) was used to quantitatively weight their influence on AIDS progression by considering these as covariables in the Cox analysis of *CCR5P* genotypes. The *CCR5* and *CCR2* effects have been confirmed in multiple studies (*8, 10, 11*), whereas the *SDF1* protection has been affirmed for the death endpoint in one study (*33*) but not in another (*24*). We have chosen to consider *SDF1-3′A/3′A* as protective because that is the observation for the cohorts considered here in both separate and combined analyses.
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28. Quantitative fluorescence-activated cell sorting (FACS) analysis was done on various subsets of PBMCs. Four-color FACS was used to quantify the amount of 2D7 binding sites on fresh, unstimulated $CD4^+$ memory and naïve T cells from individuals wild-type for the *CCR5* and *CCR2 ORF* locus. CCR5 was not expressed on naïve T cells ($CD45RO^-$) but was present on memory T cells ($CD45RO^+$). Among memory T cells, relative to the $CD62L^+$ subset, the $CD62L^-$ subset (true memory cells) expressed at least five times as much CCR5 on the cell surface. This was true regardless of the *CCR5* promoter genotype.
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36. The possibility that the AIDS acceleration effects associated with the [+.*P1*.+]/[+.*P1*.+] genotype might reflect differences in survival due to better treatments late in the epidemic is unlikely because the clinical data used here were collected from 1978 to 1996, before the wide use of highly active antiretroviral therapy (HAART). Further, the distribution of *CCR5P1/P1* accelerating genotypes was examined in different time periods of the AIDS epidemic with our patients as follows. Three Caucasian groups (combined cohort seroconverters, $N = 632$; MACS seroconverters, $N = 397$; and MHCS seroconverters, $N = 226$) were partitioned into halves and thirds on the basis of date of seroconversion. We observed a modest depletion of [+.*P1*.+]/[+.*P1*.+] homozygotes in the earlier seroconverters (9% vs. 15% in later groups; $P = 0.01$ to 0.14) in six separate tests. In addition, [+.*P1*.+]/[+.*P1*.+] seroconverters infected early survived longer than those infected later. Both of these results likely reflect depletion of the most rapid progressors from certain categories, particularly hemophiliacs, plus the higher failure rates for Epstein-Barr virus transformation (providing DNA) among rapid progressors (*35*). If affirmed, the increase of protective [+.*P1*.+]/[+.*P1*.+] genotypes in later seroconverters and the longer survival of early versus late [+.*P1*.+]/[+.*P1*.+] patients are inconsistent (in the wrong direction) with a scenario whereby more effective recent therapies influenced the genetic effects. Pre-1996 treatment with AZT (zidovudine) was not considered formally because timing, duration, and dosage were sporadic in these patients, precluding a robust analysis.
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Role of MEKK1 in Cell Survival and Activation of JNK and ERK Pathways Defined by Targeted Gene Disruption

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Targeted disruption of the gene encoding MEK kinase 1 (MEKK1), a mitogen-activated protein kinase (MAPK) kinase kinase, defined its function in the regulation of MAPK pathways and cell survival. $MEKK1^{-/-}$ embryonic stem cells from mice had lost or altered responses of the c-Jun amino-terminal kinase (JNK) to microtubule disruption and cold stress but activated JNK normally in response to heat shock, anisomycin, and ultraviolet irradiation. Activation of JNK was lost and that of extracellular signal-regulated protein kinase (ERK) was diminished in response to hyperosmolarity and serum factors in $MEKK1^{-/-}$ cells. Loss of MEKK1 expression resulted in a greater apoptotic response of cells to hyperosmolarity and microtubule disruption. When activated by specific stresses that alter cell shape and the cytoskeleton, MEKK1 signals to protect cells from apoptosis.

MEKK1 is a 196-kD protein serine-threonine kinase that has regulatory sequences for binding the small guanine nucleotide–binding proteins Ras (*1*) and Cdc42/Rac (*2*), an NH_2-terminal bidentate 14-3-3 binding site (*3*), and a putative pleckstrin homology domain. When transfected into various cell types, MEKK1 activates the JNK and ERK pathways (*4, 5*). Expression of the catalytic domain of MEKK1 preferentially activates the JNK pathway (*5*). In contrast, expression of the complete enzyme effectively activates both the JNK and ERK pathways (*6*). MEKK1 is activated through the epidermal growth factor (EGF) receptor in PC12 (*7*), Cos (*3*), and T47D cells (*8*); the formyl-Met-Leu-Phe receptor in neutrophils (*9*); and antigen ligation of the high-affinity immunoglobulin E receptor, FcεR1, in mast cells (*10*). Catalytically inactive inhibitory mutants of MEKK1 block receptor activation of both the JNK and ERK pathways (*2*), suggesting MEKK1 has the potential to regulate both MAPK pathways. MEKK1 is also a caspase-3 substrate (*11*). Cleavage of MEKK1 at Asp^{874} releases from cell membranes a 91-kD COOH-terminal kinase domain that amplifies caspase activation and induces apoptosis (*11*).

In mammalian cells, it is unprecedented for a MAPK kinase kinase to regulate two MAPK

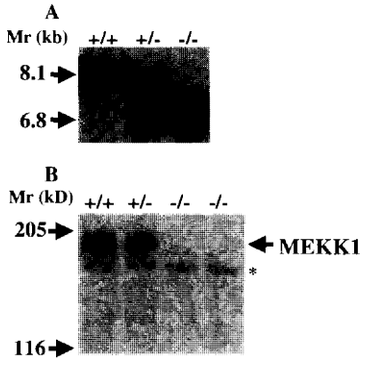

Fig. 1. Gene targeting of MEKK1. (**A**) Genomic analysis of ES cells. Genomic DNA was isolated from $MEKK1^{+/+}$, $MEKK^{+/-}$, and $MEKK1^{-/-}$ cells, digested with Eco R1, and analyzed by Southern blotting with a 5′ flanking probe. (**B**) Absence of MEKK1 protein in $MEKK1^{-/-}$ cells. Cell lysates from $MEKK1^{+/+}$, $MEKK1^{+/-}$, and $MEKK1^{-/-}$ cells were resolved by SDS-PAGE, transferred to filters, and probed with an antibody to the COOH-terminus of MEKK1. The faint band (*) is a nonspecific band that is not related to MEKK1. Four additional antisera to different MEKK1 epitopes did not detect this band in MEKK1 immunoblots. Mr, molecular mass.

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