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Genetic Variation in the *CCL18-CCL3-CCL4* Chemokine Gene Cluster Influences HIV Type 1 Transmission and AIDS Disease Progression

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CCL3 (MIP-1α), CCL4 (MIP-1β), and CCL18 (DC-CK1/PARC/AMAC-1) are potent chemoattractants produced by macrophages, natural killer cells, fibroblasts, mast cells, $CD4^+$ T cells, and $CD8^+$ T cells. CCL3 and CCL4 are natural ligands for the primary human immunodeficiency virus type 1 (HIV-1) coreceptor CCR5 and are also known to activate and enhance the cytotoxicity of natural killer cells. Genomic DNAs from >3,000 participants enrolled in five United States–based natural-history cohorts with acquired immunodeficiency syndrome (AIDS) were genotyped for 21 single-nucleotide polymorphisms (SNPs) in a 47-kb interval on chromosome 17q12 containing the genes *CCL3, CCL4,* and *CCL18*. All 21 SNPs were polymorphic in African Americans (AAs), whereas 7 of the 21 had minor-allele frequencies <0.01 in European Americans (EAs). Substantial linkage disequilibrium was observed in a 37-kb interval containing 17 SNPs where many pairwise D' values exceeded 0.70 in both racial groups, but particularly in EAs. Four and three haplotype blocks were observed in AAs and EAs, respectively. Blocks were strongly correlated with each other, and common haplotype diversity within blocks was limited. Two significant associations are reported that replicate an earlier study. First, among AA members of the AIDS Link to the Intravenous Experience cohort of injection drug users, frequencies of three correlated SNPs covering 2,231 bp in *CCL3* were significantly elevated among highly exposed, persistently HIV-1–uninfected individuals compared with HIV-1–infected seroconvertors (P = .02–.03). Second, seven highly correlated SNPs spanning 36 kb and containing all three genes were significantly associated with more-rapid disease progression among EAs enrolled in the Multicenter AIDS Cohort Study cohort (P = .01–.02). These results reiterate the importance of chemokine gene variation in HIV-1/AIDS pathogenesis and emphasize that localized linkage disequilibrium makes the identification of causal mutations difficult.

The heterogeneity among individuals in their susceptibility to HIV type 1 (HIV-1) infection and the variable rates of disease progression among HIV-1–infected individuals are the results of many factors, including viral virulence, environmental conditions, age, and host genetics. Host antiviral immunity can act at different levels, including the inhibition of viral attachment, entry, or assembly, and may include various responses to viral infection, such as cytotoxic T-cell response, the generation of noncytolytic factors such as cytokines, and antibody production.[1]

AIDS restriction genes have been defined in which allelic variation has been shown to influence infection or disease progression. These include genes encoding chemokine receptors, chemokines, human leukocyte antigen, and cytokines and genes involved with innate or acquired immunity.[2] Chemokines became prominent in HIV-1 research when the culture supernatants of $CD8^+$ T cells demonstrated HIV-1 suppressor activity,[3,4] and this activity was shown to be attributable to three CC chemokines—namely, RANTES (CCL5), MIP-1α (CCL3), and MIP-1β (CCL4).[5] Chemokines are a group of low-molecular-weight, basic, heparin-binding proteins that mediate an array of homeostatic and inflammatory processes. The amino acid sequences contain four conserved cysteine residues, and these molecules signal through receptors belonging to the G-protein–coupled seven-transmembrane domain receptor family.[6] Over 50 chemokine genes are assigned to two major subgroups: the α (or C × C) and the β (or CC) subgroups. Sixteen members of the CC gene family have been assigned to chromosome 17q12, and many of these genes exhibit extreme sequence conservation, suggesting recent duplication and selection for conserved function in their evolutionary histories.[7,8]

The *CCL3* and *CCL4* chemokine genes, along with *CCL18,* are located within 40 kb of each other. SNPs in the *CCL3* gene were evaluated in two previous HIV-1 epidemiological studies. Four *CCL3* SNPs were not significantly associated with HIV-1 infection in a Japanese population,[9] whereas a haplotype containing two *CCL3* intron 1 SNPs was associated with resistance to HIV-1 acquisition in African Americans (AAs) and with accelerated progression to AIDS in European Americans (EAs).[10] Given the

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obvious role played by CCL3 and CCL4 in HIV-1/AIDS pathogenesis and the availability of high-density SNP maps for population-based association analyses, we examined, in the present study, variation in 21 SNPs spanning 47 kb of genomic DNA containing the *CCL18*, *CCL3*, and *CCL4* genes in >3,000 participants enrolled in five United States–based longitudinal HIV-1/AIDS cohorts. Two primary questions were addressed in the study. First, what is the extent and nature of SNP and haplotype variation in and around these three genes in two racial groups? Second, do genetic variants exist in these genes that influence HIV-1 infection and/or AIDS disease progression?

Material and Methods

Participants and Clinical Outcomes

Participants were enrolled in five longitudinal cohorts. The AIDS Link to the Intravenous Experience (ALIVE) is a community-based cohort of adult injection drug users in Baltimore.[11] The racial distribution is 92.4% AA and 7.6% EA. The Hemophilia Growth and Development Study (HGDS) is a multicenter prospective study that enrolled children with hemophilia.[12] The cohort consists of 126 HIV-1–uninfected and 207 HIV-1–infected children who were exposed to HIV-1 through blood products between 1982 and 1983. The racial distribution is 72% EA, 15% Hispanic, and 11% AA. The Multicenter Hemophiliac Cohort (MHCS) is a prospective study that enrolled persons with hemophilia.[13] The racial distribution is 90% EA, 6% AA, and 3% Hispanic. The Multicenter AIDS Cohort Study (MACS) is a longitudinal study of men who have sex with men (MSM) from four U.S. cities: Chicago, Baltimore, Pittsburgh, and Los Angeles.[14,15] The racial distribution is 83.3% EA, 10% AA, and 5% Hispanic. The San Francisco City Clinic Study (SFCC) is a cohort of MSM originally enrolled in a hepatitis B study in 1978–1980.[16] The cohort consists of 211 individuals, 203 of whom are EA. The majority of subjects were enrolled into the cohorts during the following years: ALIVE, 1988–1989; MACS, 1984–1985; MHCS, 1982–1985; and SFCC, 1978–1980.

All individuals used for association analyses were (1) uninfected individuals who have undocumented levels of exposure but who

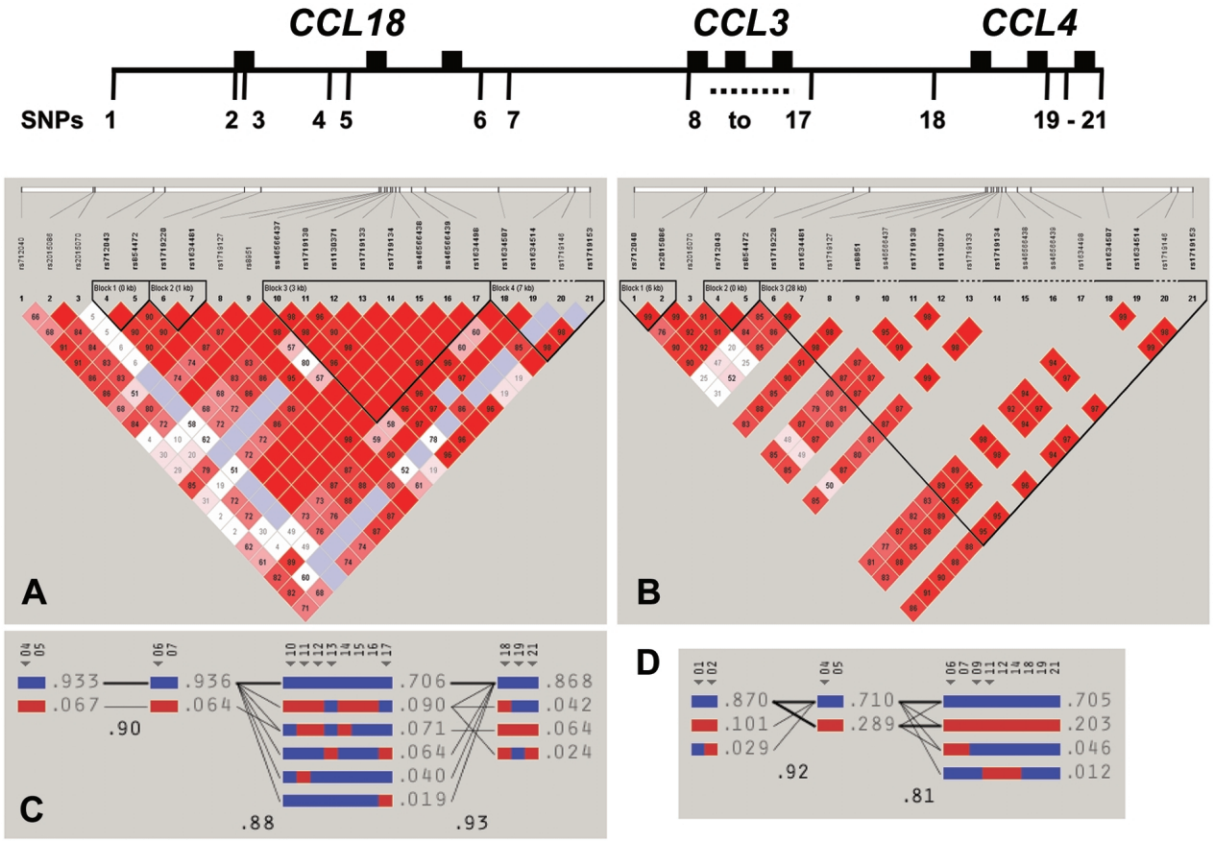

Figure 1. Molecular map of the *CCL18*, *CCL3*, and *CCL4* genes. Each gene has three exons, depicted as blackened boxes, and SNPs are numbered 1–21. The distance between SNPs 1 and 21 is 47,637 bp. Below the map are pairwise *D′* plots and haplotype blocks obtained from HAPLOVIEW for 420 AAs (*A*) and 972 EAs (*B*) (in panel *B*, seven tracks are blank because of MAFs <0.01). Dark-red squares signify high *D′* values, light-blue squares indicate high *D′* values with low LOD scores, and light-red and white squares indicate low *D′* values. Block boundaries are based on the 95% CIs for *D′*.[23] Panels C and D show the structure of haplotype blocks found in AAs and EAs, respectively. Haplotype-tagging SNPs identified by HAPLOVIEW are indicated by arrowheads. Numbers below the blocks are interblock, multiallelic *D′* values.

Table 1. MAFs and Gene Positions for 21 SNPs in the *CCL18*, *CCL3*, and *CCL4* Chemokine Genes

SNP	dbSNP	MAF[a]		Location	Position	Inter-SNP Distance[b] (bp)
		AA	EA			
1	*rs712040*	.497	.108	*CCL18* promoter	−6111 C/T	...
2	*rs2015086*	.384	.13	*CCL18* promoter	−86 C/T	6,025
3	*rs2015070*	.039	.087	*CCL18* intron 1	+81 A/G	166
4	*rs712043*	.058	.302	*CCL18* intron 1	+4965 C/T	4,884
5	*rs854472*	.062	.304	*CCL18* intron 1	+5906 A/G	941
6	*rs1719220*	.055	.277	*CCL18* 3′ UTR	+12575 A/G	6,669
7	*rs1634481*	.055	.271	*CCL3* 3′ UTR	+11730 A/G	1,398
8	*rs1719127*	.121	.004	*CCL3* 3′ UTR	+1829 A/G	9,901
9	*rs8951*	.081	.218	*CCL3* exon 3	+1685 C/T	144
10	*ss46566437*	.083	.004	*CCL3* exon 3	+1342 G/T (E79D)	343
11	*rs1719130*	.194	.24	*CCL3* intron 2	+1159 C/T	183
12	*rs1130371*	.154	.234	*CCL3* exon 2	+868 C/T (P60P)	291
13	*rs1719133*	.065	.005	*CCL3* intron 1	+740 A/G	128
14	*rs1719134*	.151	.24	*CCL3* intron 1	+459 A/G	281
15	*ss46566438*	.083	.005	*CCL3* intron 1	+113 C/T	346
16	*ss46566439*	.08	.004	*CCL3* promoter	−891 T/C	1,002
17	*rs1634498*	.077	.003	*CCL3* promoter	−2021 C/T	1,131
18	*rs1634507*	.113	.237	*CCL4* promoter	−5725 A/C	6,149
19	*rs1719144*	.054	.222	*CCL4* intron 1	+104 A/T	5,829
20	*rs1719146*	.032	0	*CCL4* exon 2	+663 C/T (T39T)	558
21	*rs1719153*	.077	.233	*CCL4* 3′ UTR	+1931 A/T	1,268

[a] MAFs were determined by genotyping 675–1,116 AAs and 978–2,054 EAs for any given SNP.
[b] Distance from the previous SNP. The total distance between SNPs 1 and 21 is 47,637 bp.

belong to an HIV-1 risk group; (2) high-risk, exposed, uninfected (HREU) individuals with documented high risk for HIV-1 exposure; (3) HIV-1–positive seroconvertors, with seroconversion dates estimated as the midpoint between the last visit with seronegative test results and the first visit with seropositive test results; or (4) seroprevalent individuals who were infected with HIV-1 at study enrollment. The HREU participants were exposed to HIV-1 by receptive anal intercourse with multiple partners,[15,16] by transfusion with nonheated units of factor VIII or IX between 1982 and 1983,[17] or by injection drug use.[11] A concise summary of these cohorts is available.[18] Over 3,000 individuals were genotyped, and the data were used to estimate allele frequencies, but genotypes from only 1,326 individuals were used in disease association analyses: 449 AAs (159 seronegative individuals and 290 seroconvertors) and 877 EAs (216 seronegative individuals and 661 seroconvertors). Since antiretroviral treatment history was unavailable for a majority of subjects, participants were censored for highly active antiretroviral therapy (HAART), instead of the effects of HAART being adjusted for in the models.[19] The censoring date was the earlier of two dates: the last recorded follow-up visit or December 31, 1995, for the MACS, MHCS, HGDS, and SFCC cohorts and the last recorded visit or July 31, 1997, for the ALIVE cohort. A later censoring date was used for the ALIVE cohort because administration of HAART was delayed.[20] The median follow-up times were 5.1, 7.3, 10.7, and 13.4 years for the ALIVE, MACS, MHCS, and SFCC cohorts, respectively.

Three separate end points reflecting advancing AIDS pathogenesis were considered for seroconvertors: (1) the Centers for Disease Control and Prevention (CDC) definition of AIDS in 1993 (AIDS-93): HIV-1 infection plus a $CD4^+$ T cell count of $<200/\mu l$ or AIDS-defining conditions[21]; (2) the CDC definition of AIDS in 1987 (AIDS-87): HIV-1 infection plus AIDS-defining illness[22]; and (3) AIDS-related death during follow-up of an HIV-1–infected patient.

Table 2. Primers and Probes and the Restriction Enzymes Used in Genotyping Assays

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

SNP Discovery and Genotyping

The *CCL18*, *CCL4*, and *CCL3* genes are located within 40 kb of one another at chromosome 17q12 (fig. 1). Each gene contains three exons and two introns.[24] SNPs were identified by using the SSCP technique, by resequencing the *CCL3* and *CCL4* genes, or by surveying the dbSNP database (build 110). For SSCP or resequencing, 12 different genomic regions covering >3,000 bp were analyzed on DNAs from 36 unrelated EAs and 36 unrelated AAs presenting with various HIV-1/AIDS phenotypes. A total of 21 SNPs were genotyped (table 1) by use of either PCR-RFLP or TaqMan allelic discrimination (Applied Biosystems) techniques (table 2).[25]

Statistical Analyses

Allele frequencies and genotype frequencies were calculated and tests for Hardy-Weinberg equilibrium were performed using SAS Genetics software (SAS Institute). Unphased diploid genotype data were partially phased by use of PHASE,[26] and the output from PHASE was input into HAPLOVIEW[27] for pairwise D' and r^2 calculation, haplotype estimation, and haplotype-block construction. Comparisons of allele, genotype, and haplotype frequencies between the seronegative HREU individuals and the HIV-1–infected seroconvertors were done with Fisher's exact test or logistic regression by use of SAS. Cox proportional hazards regres-

Table 3. Survival Analyses of Progression to Three AIDS End Points for *CCL18, CCL3,* and *CCL4* SNPs and One Haplotype

	AIDS-93			AIDS-87			Death		
Group and SNP or Haplotype	NE	RH (95% CI)	*P*	NE	RH (95% CI)	*P*	NE	RH (95% CI)	*P*
AAs in all cohorts (n = 290):	…	…	…	…	…	…	…	…	…
rs712040	124	1.01 (.60–1.52)	.96	57	.86 (.49–1.52)	.6	33	1.37 (.61–.50)	.45
rs2015086	125	1.09 (.75–1.57)	.66	59	.95 (.56–1.61)	.84	32	1.12 (.55–2.28)	.76
rs2015070	125	1.11 (.55–2.22)	.77	59	.79 (.28–2.23)	.65	34	.71 (.17–3.06)	.65
rs712043	125	1.18 (.72–1.94)	.5	59	1.09 (.53–2.25)	.81	34	.75 (.26–2.15)	.59
rs854472	128	1.11 (.68–1.82)	.68	61	1.03 (.50–2.12)	.93	35	.73 (.25–2.07)	.55
rs1719220	130	1.26 (.75–2.11)	.39	63	1.18 (.55–2.53)	.68	36	.98 (.34–2.81)	.97
rs1634481	129	.06 (.70–2.01)	.52	63	1.10 (.51–2.38)	.8	36	.92 (.32–2.65)	.88
rs1719127	131	.96 (.62–1.51)	.87	62	.84 (.43–1.62)	.6	35	1.18 (.53–2.64)	.68
rs8951	131	1.34 (.85–2.11)	.2	61	1.26 (.66–2.43)	.48	35	1.69 (.75–3.81)	.21
ss46566437	132	.97 (.58–1.61)	.9	62	.77 (.36–1.65)	.5	35	.77 (.27–2.19)	.62
rs1719130	125	1.3 (.89–1.94)	.18	57	1.27 (.73–2.20)	.4	32	1.29 (.62–2.68)	.49
rs1130371	131	1.34 (.91–1.94)	.14	62	1.21 (.69–2.11)	.5	35	.90 (.41–1.95)	.78
rs1719133	132	.91 (.52–1.59)	.74	62	1.09 (.51–2.32)	.82	35	1.16 (.45–3.02)	.76
rs1719134	125	1.25 (.83–1.88)	.29	56	.96 (.51–1.82)	.91	32	.79 (.32–1.96)	.62
ss46566438	129	.90 (.53–1.53)	.69	60	.85 (.40–1.81)	.67	35	.80 (.28–2.28)	.68
ss46566439	130	.92 (.54–1.56)	.75	60	.65 (.28–1.52)	.32	35	.91 (.32–2.59)	.86
rs1634498	129	1.02 (.62–1.69)	.93	60	1.13 (.56–2.26)	.74	34	1.29 (.53–3.16)	.58
rs1634507	130	1.53 (1.01–2.34)	.05*	60	1.33 (.71–2.50)	.37	35	.64 (.24–1.69)	.36
rs1719144	129	1.52 (.91–2.52)	.11	60	1.34 (.61–2.95)	.47	34	.66 (.19–2.31)	.52
rs1719146	126	2.37 (1.11–5.06)	.03*	59	2.72 (.99–7.49)	.05*	34	1.39 (.30–6.37)	.67
rs1719153	129	1.55 (.96–2.48)	.07	62	1.60 (.81–3.13	.17	35	.71 (.24–2.13)	.54
Haplotype *[block 3-.071]*	123	1.37 (.77–2.34)	.21	60	1.19 (.55–2.47)	.38	33	.84 (.33–2.26)	.51
rs1719146	104	2.38 (1.12–5.04)	.02*	46	2.72 (.97–7.56)	.06	22	2.17 (.47–9.97)	.32
EAs in all cohorts (n = 661):	…	…	…	…	…	…	…	…	…
rs712040	406	.96 (.75–1.23)	.75	293	.95 (.71–1.28)	.74	250	.93 (.66–1.29)	.65
rs2015086	413	.96 (.76–1.21)	.72	299	.98 (.75–1.29)	.89	256	.97 (.72–1.31)	.85
rs2015070	406	.90 (.69–1.17)	.43	293	.80 (.58–1.12)	.19	250	.82 (.57–1.17)	.27
rs712043	411	1.09 (.89–1.32)	.4	298	1.16 (.92–1.46)	.22	255	1.08 (.84–1.39)	.55
rs854472	408	1.11 (.91–1.35)	.3	294	1.21 (.96–1.53)	.11	250	1.12 (.87–1.44)	.38
rs1719220	410	1.04 (.85–1.27)	.71	298	1.09 (.86–1.37)	.48	253	.97 (.75–1.24)	.79
rs1634481	396	1.06 (.87–1.30)	.55	286	1.14 (.90–1.44)	.29	240	.99 (.77–1.29)	.96
rs1719127[a]	…	…	…	…	…	…	…	…	…
rs8951	409	1.15 (.95–1.40)	.15	298	1.30 (1.03–1.63)	.03*	262	1.06 (.83–1.36)	.64
ss46566437	…	…	…	…	…	…	…	…	…
rs1719130	400	1.24 (1.01–1.51)	.04*	289	1.39 (1.10–1.76)	.01*	250	1.25 (.97–1.60)	.09
rs1130371	414	1.17 (.97–1.43)	.11	302	1.33 (1.06–1.67)	.01*	263	1.15 (.90–1.47)	.26
rs1719133	…	…	…	…	…	…	…	…	…
rs1719134	418	1.18 (.98–1.44)	.09	304	1.34 (1.07–1.68)	.01*	264	1.17 (.92–1.49)	.21
ss46566438	…	…	…	…	…	…	…	…	…
ss46566439	…	…	…	…	…	…	…	…	…
rs1634498	…	…	…	…	…	…	…	…	…
rs1634507	405	1.14 (.93–1.38)	.21	297	1.23 (.98–1.54)	.08	257	1.07 (.83–1.37)	.61
rs1719144	392	1.20 (.98–1.47)	.07	287	1.32 (1.05–1.67)	.02*	252	1.09 (.85–1.41)	.49
rs1719146	…	…	…	…	…	…	…	…	…
rs1719153	409	1.19 (.98–1.45)	.08	297	1.33 (1.06–1.68)	.01*	253	1.20 (.94–1.54)	.15
EAs in MACS cohort (n = 403):	…	…	…	…	…	…	…	…	…
rs712043	248	1.14 (.89–1.47)	.3	183	1.38 (1.02–1.85)	.04*	159	1.18 (.86–1.62)	.31
rs854472	248	1.15 (.89–1.48)	.28	181	1.41 (1.05–1.91)	.02*	156	1.20 (.87–1.65)	.27
rs1634481	246	1.08 (.83–1.39)	.57	179	1.27 (.94–1.71)	.12	153	1.01 (.73–1.39)	.95
rs8951	243	1.21 (.94–1.57)	.14	182	1.43 (1.06–1.91)	.02*	162	1.12 (.82–1.53)	.46
rs1719130	241	1.19 (.92–1.54)	.18	179	1.40 (1.04–1.88)	.02*	158	1.22 (.89–1.68)	.21
rs1130371	245	1.22 (.95–1.57)	.13	184	1.41 (1.06–1.89)	.02*	162	1.15 (.85–1.57)	.37
rs1719134	250	1.22 (.95–1.57)	.11	187	1.42 (1.07–1.90)	.02*	164	1.19 (.87–1.62)	.28
rs1634507	238	1.19 (.92–1.54)	.18	180	1.32 (.99–1.78)	.06	157	1.12 (.82–1.54)	.47
rs1719144	228	1.19 (.92–1.55)	.19	172	1.42 (1.05–1.91)	.02*	153	1.13 (.82–1.56)	.44
rs1719153	247	1.22 (.95–1.57)	.12	183	1.45 (1.08–1.94)	.01*	158	1.26 (.92–1.73)	.15

NOTE.—End points were assessed using Cox proportional hazards regression under a dominant genetic model (results include *CCL5* covariates listed in the "Material and Methods" section). n = number of seroconvertors; NE = number of events. *P* values ≤.05 are indicated by an asterisk (*).

[a] Results not reported for seven SNPs with near-zero MAFs in EAs.

sion and Kaplan-Meier survival statistics assessed rates of disease progression among seroconvertors. Regression and survival analyses were performed twice—once by using each SNP or haplotype alone and once by including three SNPs from *CCL5* (*RANTES*) as covariates: *RANTES-3′ 222, RANTES-In1.1* (*rs2280789*), and *RANTES-(−403)* (*rs2107538*).[28,29] Participants were stratified by sex (results are shown for males only) and age at HIV-1 seroconversion: <20 years, 20–40 years, and >40 years. The results stratified by age were included in the model and appear in table 3.

Results

Twenty-one SNPs covering a 47,637-bp region containing *CCL18, CCL3,* and *CCL4* (fig. 1) were genotyped in up to

Table 4. A Comparison of *CCL18, CCL3,* and *CCL4* Dominant Genotype Frequencies between Seroconvertors (HIV+) and HREU Participants (HIV-1 Seronegative)

	AAs in All Cohorts				EAs in All Cohorts[a]			
SNP	HIV+ (n = 290)	HIV-1 Seronegative (n = 79)	OR (95% CI)	P	HIV+ (n = 661)	HIV-1 Seronegative (n = 128)	OR (95% CI)	P
rs712040	.714	.708	1.03 (.58–1.82)	1	.189	.183	1.04 (.64–1.70)	1
rs2015086	.599	.569	1.13 (.67–1.90)	.69	.236	.227	1.05 (.67–1.66)	.91
rs2015070	.076	.118	.61 (.27–1.39)	.25	.165	.164	1.00 (.60–1.69)	1
rs712043	.129	.149	.84 (.39–1.80)	.69	.498	.461	1.15 (.79–1.69)	.51
rs854472	.135	.143	.93 (.44–1.98)	.85	.505	.461	1.19 (.82–1.75)	.38
rs1719220	.124	.127	.98 (.46–2.07)	1	.459	.448	1.05 (.71–1.54)	.85
rs1634481	.125	.115	1.10 (.51–2.39)	1	.453	.437	1.06 (.73–1.57)	.77
rs1719127	.207	.278	.68 (.38–1.19)	.22	…	…	…	…
rs8951	.176	.127	1.47 (.71–3.04)	.39	.396	.353	1.20 (.82–1.77)	.38
ss46566437	.153	.241	.57 (.31–1.04)	.09	…	…	…	…
rs1719130	.345	.329	1.08 (.62–1.86)	.89	.398	.423	.90 (.61–1.32)	.62
rs1130371	.295	.304	.96 (.56–1.65)	.89	.417	.409	1.03 (.71–1.50)	.92
rs1719133	.115	.179	.60 (.30–1.18)	.13	…	…	…	…
rs1719134	.263	.316	.77 (.45–1.33)	.39	.414	.411	1.01 (.70–1.46)	1
ss46566438	.151	.244	.55 (.30–1.01)	.06	…	…	…	…
ss46566439	.143	.241	.53 (.29–.97)	.04*	…	…	…	…
rs1634498	.154	.208	.69 (.37–1.31)	.3	…	…	…	…

NOTE.—ORs, 95% CIs, and P values were obtained using Fisher's exact test. P values $\leq$.05 are indicated by an asterisk (*). n = number of individuals examined.

[a] Results are not reported for seven SNPs with near-zero MAFs. Individuals homozygous for the *CCR5Δ32* allele[30] were not included.

3,158 participants. Three SNPs occurred within coding regions as follows: (1) *ss46566437* at +1342G→T changes amino acid position 79 (E79D) in exon 3 of the *CCL3* gene, (2) *rs1130371* at +868C→T is a silent substitution (P60P) in exon 2 of the *CCL3* gene, and (3) *rs1719146* at +663C→T is a silent substitution (T39T) in exon 2 of the *CCL4* gene. All other SNPs were noncoding and were found in promoter regions, introns, and intergenic areas (fig. 1 and table 1). Of the 21 SNPs, 7 had minor-allele frequencies (MAFs) <0.01 in EAs and were excluded in all subsequent analyses of EAs, whereas all 21 SNPs had MAFs >0.03 in AAs and were retained in analyses of AAs (table 1).

Linkage Disequilibrium (LD) and Haplotype Analyses

Pairwise D' values for AAs and EAs are shown (fig. 1*A* and 1*B*). In AAs, the three SNPs at both (5′ and 3′) ends of the region had much lower D' values than did the 15 SNPs in the middle. Similarly, in EAs, the three SNPs at the 5′ end had lower D' values than the remaining 11 SNPs. In addition, 16 (7.6%) of 211 pairwise r^2 values exceeded 0.70 in AAs, whereas 30 (33%) of 91 pairwise r^2 values exceeded 0.70 in EAs. These higher r^2 values in EAs are reflected by the limited range in MAFs (0.218–0.304) for all but the three SNPs at the 5′ end, whereas MAFs in AAs showed a greater range across the entire region (0.032–0.497) (table 1).

Haplotype-block analysis identified four blocks in AAs, which were <1 kb, 1 kb, 3 kb, and 7 kb in size, and three blocks in EAs, which were 6 kb, <1 kb, and 28 kb in size (fig. 1*C* and 1*D*). Diversity within each block was limited; only block 3 in AAs had more than two common haplotypes with frequencies exceeding 5%. In addition, multiallelic D' values between blocks ranged from 0.81 to 0.93, indicating extensive disequilibrium between blocks in both AAs and EAs. Although there are recombinant haplotypes in AA blocks 3 and 4 and in EA block 3, only one common haplotype (frequency >0.05) contains information that is not available from analyzing the individual SNPs. This haplotype is found in AA block 3 at a frequency of 0.071 and is named "AA haplotype *[block 3-0.071]*." This haplotype is a recombinant containing only a portion of the minor alleles from *rs1719130, rs1130371,* and *rs1719134* (fig. 1*C*). On the other hand, all other common haplotypes contain all minor alleles for at least one SNP and are thus redundant with that SNP. For example, AA haplotype *[block 3-0.090]* contains a portion of alleles from three SNPs but contains all alleles from SNPs *ss46566437, ss46566438,* and *ss46566439* (fig. 1*C*).

Disease Associations

The influence of each SNP and the single unique haplotype—AA haplotype *[block 3-0.071]*—on HIV-1 transmission was examined by comparing HREU individuals with HIV-1–infected seroconvertors after stratifying by race (tables 4 and 5). Among AAs from all cohorts combined, *ss46566439,* occurring in *CCRL3,* had significantly elevated genotype frequency in the 79 HREU individuals (frequency 0.241) compared with the 296 HIV-1–positive seroconvertors (frequency 0.143) (P = .04). To minimize

Table 5. A Comparison of *CCL18, CCL3,* and *CCL4* Dominant Genotype and Haplotype Frequencies between Seroconvertors (HIV+) and HREU Participants (HIV-1 Seronegative)

	AAs in ALIVE Cohort[a]				AAs in All Cohorts				EAs in MHCS Cohort			
SNP or Haplotype	HIV+	HIV-1 Seronegative	OR (95% CI)	*P*	HIV+ (*n* = 296)	HIV-1 Seronegative (*n* = 79)	OR (95% CI)	*P*	HIV+ (*n* = 170)	HIV-1 Seronegative (*n* = 25)	OR (95% CI)	*P*
ss46566437	.143	.26	.47 (.25–.90)	.03*	…	…	…	…	…	…	…	…
ss46566438	.145	.264	.48 (.25–.89)	.03*	…	…	…	…	…	…	…	…
ss46566439	.135	.26	.44 (.23–.84)	.02*	…	…	…	…	…	…	…	…
Haplotype												
[block 3-.071]	.126	.096	1.31 (.49–3.52)	.61	.126	.096	1.35 (.57–3.21)	.49	…	…	…	…
rs2105086	…	…	…	…	…	…	…	…	.176	.4	.32 (.13–.78)	.02*

NOTE.—ORs, 95% CIs, and *P* values were obtained using Fisher's exact test for SNPs and logistic regression for the haplotype. *P* values ⩽.05 are indicated by an asterisk (*). *n* = number of individuals examined.

[a] HIV+, *n* = 238 for SNPs and 296 for the haplotype; HIV-1 Seronegative, *n* = 73 for SNPs and 79 for the haplotype.

inherent variability resulting from the combining of cohorts, the predominant AA cohort (the injection-drug-using ALIVE cohort) was considered separately. Among AAs in the ALIVE cohort, three *CCL3* SNPs—*ss46566437, ss46566438,* and *ss46566439*—were significantly elevated in frequency in the 73 HREU individuals compared with the 238 HIV-1–infected seroconvertors (*P* = .02–.03). These three SNPs are found together on one AA haplotype, *[block 3-0.090]*, which spans 2,231 bp (fig. 1 and table 1). Among EAs from all cohorts combined, no SNPs were significant when the 128 HREU individuals were compared with the 661 HIV-1–positive seroconvertors. When the EAs were stratified by cohort, the dominant *rs2015086* genotype had a frequency of 0.40 in the 25 hemophilic HREU individuals in the MHCS cohort and a frequency of 0.18 in the 170 seroconvertors in the MHCS cohort (*P* = .02) (tables 4 and 5).

The influence of genetic variation in each SNP on disease progression was assessed. Cox proportional hazards regression, including *CCL5* covariates, showed SNPs *rs1634507* and *rs1719146* to be significantly associated with more-rapid disease progression (to AIDS-93 or AIDS-87) when 290 AAs from all cohorts were combined (*P* = .03–.05) (table 3). Among all AAs, *rs1719146* was significantly associated (relative hazard [RH] of 2.97; *P* = .03) when the *CCL5* covariates were not included (data not shown). When the 238 members of ALIVE were examined separately, only *rs1719146* was significantly associated (*P* = .02). Cox regression also found six SNPs to be significantly associated with more-rapid progression to AIDS-87 and one to AIDS-93, when 661 EAs from all cohorts combined were examined with *CCL5* covariates included (*P* = .01–.04) (table 3). Five of the six SNPs associated with AIDS-87 are together on a single EA haplotype, *[block 3-0.203],* that spans 28,952 bp. The same six SNPs were significant (RH = 1.29–1.39; *P* = .005–.03) among all EAs when the *CCL5* covariates were not included (data not shown). Inspection of the three major EA cohorts indicated that these disease-accelerating effects are largely attributable to the MACS cohort rather than the MHCS, HGDS, or SFCC cohort (table 3). Eight SNPs were significantly associated (*P* = .01–.04) with AIDS-87 in the MACS cohort, whereas none was associated in the MHCS, HGDS, or SFCC cohort.

The results presented in table 3 were confirmed by Kaplan-Meier survival analyses without *CCL5* covariates, in which 10 SNPs were associated with more-rapid disease progression in all EAs, including 9 SNPs in the MACS cohort (results not shown). As illustrated by the graphs in figure 2, the rate of AIDS-87 disease progression is increased among all EAs carrying one (C/T) or two (T/T) copies of the variant *rs1719130* allele compared with those carrying the wild-type (C/C) genotype (fig. 2*A*) and is increased among individuals in the MACS cohort carrying one (A/T) or two (T/T) copies of the variant *rs1719153* allele compared with those homozygous (A/A) for the wild-type allele (fig. 2*B*).

Discussion

This study evaluated the role of genetic variation in 21 SNPs in the *CCL18, CCL3,* and *CCL4* chemokine genes in HIV-1 infection and AIDS disease progression in five U.S. natural-history AIDS cohorts. There was extensive LD in the 47,637 bp surveyed in both EAs and AAs but particularly in EAs, among whom limited allelic and haplotype diversity was found. Four statistically significant associations with HIV-1 transmission or AIDS progression were identified, two of which replicate results of an earlier study. Importantly, replication has recently been reported to be the "gold standard" in genetic association studies.[31] We further discuss the two replicated associations.

The first was a protective genetic influence on HIV-1 infection for three correlated *CCL3* SNPs (*ss46566437, ss46566439,* and *ss46566438,* located on one haplotype covering 2,231 bp) in AAs from the ALIVE cohort. Although *ss46566437* is a nonsynonymous (E79D) substitution, we have no evidence indicating that it is a functional mutation. These results confirm and extend those of Gonzalez et al.,[10] who reported a significantly elevated frequency of the *ss46566438*-containing haplotype in HIV-1–negative AA subjects compared with HIV-1–positive AA

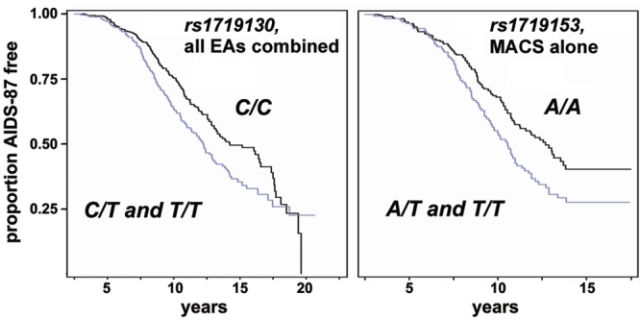

Figure 2. Kaplan-Meier survival curves showing the dependence of AIDS-87 progression (the proportion surviving who did not reach the disease end point) on the dominant *rs1719130* genotype in all EAs (*A*) and the dominant *rs1719153* genotype in the MACS cohort (*B*). *A,* The black curve (C/C) represents all individuals homozygous for the wild-type allele, and the blue curve (C/T and T/T) includes individuals with one or two copies of the minor allele. The total number of individuals was 636; 291 developed AIDS, and 345 were censored. RH = 1.50; log-rank $P = .0065$; Wilcoxon $P = .0023$; −2log(likelihood ratio) $P = .009$. *B,* The black curve (A/A) represents individuals carrying two copies of the wild-type allele, and the blue curve (A/T and T/T) represents individuals with one or two copies of the variant allele. The total number of individuals was 402; 184 developed AIDS, and 218 were censored. RH = 1.51; log-rank $P = .0072$; Wilcoxon $P = .0159$; −2log(likelihood ratio) $P = .0149$. RH values were obtained from Cox proportional hazards regression, and the *P* values were obtained from the Kaplan-Meier survival analyses.

subjects. The protective effect found in our study was slightly stronger for the ALIVE cohort alone (odds ratio [OR] 0.47) than for all AAs combined (OR 0.57). This may be a result of mode of infection, sampling artifacts, or population stratification. ALIVE is an injection-drug-user cohort from Baltimore. The second largest population of AA participants consists of 45 seroconvertors from the MACS, a cohort of homosexual men from four American cities.[18] It is well established that AAs are admixed and that different populations may have different ancestral African and European genetic contributions.[32] It is thus expected that different genetic backgrounds will reveal varying susceptibility to infectious agents.

Second, we found that each of seven highly correlated SNPs was associated with more-rapid disease progression in EAs. These SNPs span 36,562 bp and contain all three genes. Given the extensive disequilibrium in the region, it is not possible to identify a particular SNP or even a single gene as causative. Although *CCL18* has not yet been implicated in HIV-1/AIDS pathogenesis and its receptor is not known,[33] the present genetic analysis cannot rule out this gene as a candidate for modulating HIV-1–related pathogenesis. Furthermore, pairwise protein alignment scores obtained using CLUSTALW[34] were 54.4 for CCL3 and CCL4, 59.6 for CCL3 and CCL18, and 40.5 for CCL4 and CCL18. The high relative score for alignment between CCL3 and CCL18 suggests that there could be some redundancy in receptor binding between these two ligands. Importantly, this disease-accelerating association supports an earlier study in which borderline statistical significance ($P = .03$–$.09$) in Kaplan-Meier survival analysis of an EA population was reported for *rs1719134*.[10] Interestingly, the level of LD in the present study is comparable to what was reported for the *CCL2-CCL7-CCL11* chemokine gene cluster, which is 1.8 Mb upstream of the *CCL18-CCL3-CCL4* cluster.[25] In that region, a 31-kb haplotype containing all three genes was associated with decreased susceptibility to infection among EAs. Similar to the findings here, a single gene or SNP could not be singled out as being responsible for the association, given the observed extent of LD.

Although significant Cox regression *P* values were observed for EAs in the MACS cohort but not in the other cohorts, the RH values for these cohorts overlapped each other. Thus, the smaller *P* values for the MACS EAs may be influenced by the larger sample size. The MACS and SFCC cohorts consist of men who became infected with HIV-1 through unprotected anal intercourse, whereas the MHCS participants have hemophilia and were exposed to HIV-1–contaminated clotting factor; thus, route of infection does not seem to explain differences between cohorts in the association results. On the other hand, the DNA samples used in this study from the MHCS and the SFCC cohorts are enriched for long-term nonprogressors and thus represent a nonrandom segment of the population surviving with HIV-1 infection, whereas the MACS samples are a broader representation of the infected population.[18] In other words, if people developing AIDS early after infection had been recorded by the MHCS and SFCC cohorts, then perhaps their disease-progression profiles would be more similar to that of the MACS cohort.

CCR5 binding ligands such as CCL3, CCL4, and CCL5 can limit HIV-1 infection via at least three different mechanisms. After a ligand binds to its receptor, the viral envelope protein is blocked from attaching to the cell. A second possibility is that the chemokines induce endocytosis of the receptor, thus limiting availability of the receptor to virus.[35,36] It has also been suggested that chemokines can induce receptor dimerization that subsequently prevents viral proteins from interacting with the cellular membrane.[37] Further, it has been shown that the ability of CCL5 to limit HIV-1 infection can vary between cell types.[38] In addition, gene-by-gene and gene-by-environment interactions certainly act on HIV-1/AIDS pathology, as do various social and environmental variables.[39] The idea that certain genotypes act only under specific environmental conditions or that a given genotype may produce different phenotypes in different environments is a distinct possibility.[31] These interactions are more complex than was initially anticipated, reinforcing the need for further study and emphasizing the importance of replication among studies with adequate power.

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