

MCP-1-MCP-3–Eotaxin gene cluster influences HIV-1 transmission

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Background: *MCP-1* (*CCL2*), *MCP-3* (*CCL7*), and *eotaxin* (*CCL11*) are genes for CC chemokines clustered on the long arm of chromosome 17. Previous studies have implicated these chemokines in monocyte recruitment, viral replication, and anti-HIV cytotoxic T cell responses. An epidemiological analysis identified genetic variants influencing HIV-1 transmission and disease progression.

Methods: Genomic DNA from over 3000 participants enrolled in five natural history cohorts in the United States were analyzed. Nine single nucleotide polymorphisms (SNP) covering 33 kb containing these three genes were genotyped using the polymerase chain reaction. Distortions in allele, genotype, and haplotype frequencies were assessed with respect to HIV-1 transmission and rates of disease progression using categorical and survival analyses.

Results: Extensive linkage disequilibrium was observed. Three SNP (–2136T located in the *MCP-1* promoter region, 767G in intron 1 of *MCP-1*, and –1385A in the *Eotaxin* promoter) were nearly always found together on a 31 kb haplotype (H7) containing the three genes. Frequencies of the three variants and the H7 haplotype were significantly elevated (odds ratio, 0.6; $P = 0.005–0.01$) in uninfected European-Americans repeatedly exposed to HIV-1 through high-risk sexual behavior or contaminated blood products.

Conclusions: Although the extensive linkage disequilibrium precludes positive identification of the causal variant, the results suggest that genetic variation in the H7 region influences susceptibility to HIV-1 infection. Since these chemokines do not bind the primary HIV-1 coreceptors CCR5 or CXCR4, the observed influence on transmission may result from activation of the immune system in response to infection rather than receptor blockage.

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Introduction

Heterogeneity in the clinical outcomes of individuals

exposed to and infected with HIV-1 may be attributable to various factors including host genetic variation, viral strain heterogeneity, and environmental influ-

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ences. Epidemiological surveys have identified genetic variants in at least 11 human genes, including those for several chemokines and their receptors, that influence HIV-1 infection and/or AIDS disease pathogenesis (summarized by O'Brien *et al.* [1]).

The genes for the CC chemokines MCP-1, MCP-3, and eotaxin reside within 33 kb on the long arm of chromosome 17. These chemokines are secreted by a variety of cells including monocytes, macrophages, intestinal epithelial cells, lymphocytes, and endothelial cells and control the migration of monocytes, basophils, eosinophils, and others [2]. The ligands encoded by these genes bind CCR2 and CCR3. Although these two receptors bind HIV-1 *in vitro*, there is little evidence that they serve as HIV-1 coreceptors *in vivo*. However, there are a number of studies implicating MCP-1, MCP-3, and eotaxin in HIV-1/AIDS pathogenesis. Immature dendritic cells exhibited potent chemotaxis in response to MCP-1 [3,4] and MCP-3 [5], suggesting that cellular differentiation and migration may be affected by these chemokines. It has also been reported that HIV-1-infected macrophages upregulate the expression of MIP-1 α , MIP-1 β [6,7], and MCP-1 [8]. Since these proteins are potent chemoattractants for T cells and monocytes, this mechanism may enable further viral propagation. Additionally, replication in peripheral blood mononuclear cells from HIV-1-infected individuals was suppressed by RANTES and MIP-1 α and enhanced by MCP-1 [9]. Both inhibitory [9] and stimulatory [10] effects have been reported for MCP-3. Finally, one study has shown that eotaxin and MCP-3 stimulated anti-HIV cytotoxic T cells [11].

Given their importance in HIV-1/AIDS pathogenesis, the present study is the first comprehensive epidemiological analysis of genetic variation in the *MCP-1*, *MCP-3*, and *eotaxin* genes. Nine common single nucleotide polymorphisms (SNP) in and around these genes were examined among subjects enrolled in five natural history cohorts in the United States to assess their association with HIV-1 transmission.

Methods

Study participants

Participants were from five longitudinal cohorts: AIDS Link to the Intravenous Experience (ALIVE; [12]), Hemophilia Growth and Development Study (HGDS; [13]), Multicenter Hemophilia Cohort (MHCS; [14]), Multicenter AIDS Cohort Study (MACS; [15,16]), and San Francisco City Clinic Study (SFCC; [17]). Participants were classified as HIV-1 seroconverters (infected after study enrollment), HIV-1 seroprevalents (infected at enrollment), and seronegative, based on HIV-1

antibody testing. The seroconversion date was estimated as the midpoint between the first positive HIV-1 and last negative antibody test dates. The high-risk exposed, uninfected (HREU; $n = 247$) group contained homosexual men exposed through receptive anal intercourse with multiple partners [15], hemophiliacs who received contaminated factor VIII prior to the initiation of heat treatment in 1984 [18], and needle-sharing injecting drug users [19]. A total of 2012 European-Americans (EA: 534 seronegatives, 878 seroprevalents, and 600 seroconverters) and 1052 African-Americans (AA: 249 seronegatives, 579 seroprevalents, and 224 seroconverters) were genotyped for nine SNP.

Single nucleotide polymorphisms discovery

The *MCP-1*, *MCP-3*, and *eotaxin* genes all contain the standard chemokine gene three exon, two intron genomic structure [20] and are located within a 33 kb region on bacterial artificial chromosome (BAC) clone hRPK.215_E_13 (GenBank AC005549). Six to eight pairs of polymerase chain reaction (PCR) primers around each of the *MCP-1* and *eotaxin* genes were used in the single-strand conformational polymorphism (SSCP) assay to identify six SNP in DNA from 144 unrelated individuals (procedure described in Modiet *et al.* [21]). SNP -3306A/G upstream of *MCP-3* was discovered by examining differences between GenBank sequences X95070 and AC005549.

Genotyping

Six of the nine SNP were genotyped using the 5' nuclease or Taq-Man allelic discrimination technique, while three used the PCR restriction fragment length (PCR-RFLP) method. PCR primers and Turbo (propyne T) probes for -2136A were AAGATCTCAG CATCTTTCAGCTTGT and CCTAGGCCATCTC ACCTCATCTT, 5'-6FAM-TCTCTCTAATCTGT AGTGCATCCT-TAMARA, and 5'-VIC-CTCTCT CTAATCAGTTAGTGCATCCT-TAMARA. PCR primers and probes for -362G were AAGCTGGC AGCGAGCCT and GCCCAGACTGACCACAGA TGT, 6-FAM-CAGTTTTTCGCTTTCAGAGAAAGC AGAATCCT-TAMARA, and 5'-VIC-CAGTTTTTC GCTTCACAGAAAGCAGAATCC-TAMARA. PCR primers and probes for 767G were CACATTCTA GCTCTGAGGTATAGGCA and AGGATGAACT GGATTATTCTGATCTTAAG, 5'-6FAM-TTAATG AGCTCTTCTCTTCTCCTGCCTGC-TAMARA, and 5'-VIC-TAATGAGCTCTTTGTCTTCTCCTG CCTGC-TAMARA. PCR primers and Turbo (propyne T) probes for -3306G were TTCCAAGAC ATTTGGTCTT and TTCAGGTAATGGTCCC ATCCAT, 5'-6FAM-AGTCTGTTTTACATACCT CAT-TAMARA, and 5'-VIC-AGTCTGTTTTACG TACCTCA-TAMARA. PCR primers and probes for -386G were AGGAAGGTTCTTAGATCGACT CATC and TGGTTATGAAATGAAGTTAAACAT CTGTA, 5'-6FAM-TGCTCCTTTCCCCAACTAC

AGGTGTTTC-TAMARA, and 5'-VIC-TGCTCC TTTCCCCGACTACAGGTGTT-TAMARA. PCR primers and probes for Ala23Thr (67A) were TTC TGTGGCTGCTGCTCATAG and GTAGCTCTG GAGGTGGTTACCTTAC, 5'-6FAM-AGGGGCTC GCTGGGCCA-TAMARA, and 5'-VIC-CAGGGGC TCACTGGGCCAG-TAMARA. PCR amplification used ABI Taq-Man buffer, and cycling conditions, included a initial denaturation at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 62°C for 1 min.

The –2578A SNP (CAGCT(G/A)TCACT) used primers GCCAGCACTGACCTCCCA and ACAGGG AAGGTGAAGGGTATGA to produce a 120 bp product. *PvuII* digestion yielded fragments of 120 bp (common allele) and 67 and 53 bp (variant allele). The 903C SNP (TGCTG(C/T)TATAA) used primers TCCTCATGACTCTTTTCTGC and GAGGCTTG TCCCTTGCTCCAC to produce a 263 bp product. *Fnu4HI* digestion yielded fragments of 109, 91, and 63 bp (common allele) and 172 and 91 bp (variant allele). The –1385A SNP (CCACC(G/A)GGAAT) used primers TATCCTGCTCTTACCCTAGC and GGCACAGGATTAGAGACAGC to produce a 279 bp product. *MspI* digestion yielded fragments of 279 bp (common allele) and 153 and 126 bp (variant allele).

Statistical analyses

Genotype and haplotype frequencies were compared between HREU and HIV-1-infected seroconverters using both categorical analyses (two-tailed Fisher's exact test) and logistic regression using a dominant genetic model. Kaplan–Meier non-parametric survival statistics and Cox proportional hazards regression (SAS Institute software, Cary, North Carolina, USA) assessed rates of disease progression among HIV-1-infected seroconverters. Three endpoints reflecting advancing HIV-1 disease progression were evaluated: time to $< 200 \times 10^6$ cells/l for CD4 cells, AIDS-1987 (as defined by the Centers for Disease Control and Prevention [22]), and AIDS-related death during follow-up for an HIV-1-infected participant. Cox analyses were performed both unadjusted (using the genetic factor by itself) and adjusted (considering the genotype or haplotype as a covariate along with additional AIDS-modifying genetic factors. For EA, these were *CCR5-Δ32*, *CCR2-64I*, *SDF1-3'A*, *HLA-B*27*, *HLA-B*57*, *HLA-B*35Px*, and HLA class I zygosity; for AA, they were *HLA-B*57*, *HLA-B*35Px*, and HLA class I zygosity [23–27]. Participants were stratified by sex (7.6% female) and age at seroconversion: 0 to < 20 , 20–40, and over 40 years. All participants were censored as of December 1996, prior to use of highly active antiretroviral therapy (HAART). Haplotypes were estimated in compound heterozygotes using the expectation–maximization (E–M) algorithm [28].

Results

Nine common SNP spanning the *MCP-1–MCP-3–eotaxin* gene region (33 000 bp) were genotyped in 3064 human DNA samples from five AIDS cohorts. The nine SNP include five newly discovered variants, one obtained from aligning GenBank sequences and three previously described [29,30] (Fig. 1). Three of the SNP occurred within coding regions (767G, 903T, Ala23Thr), but only *eotaxin* Ala23Thr altered the amino acid sequence (Fig. 1). The others occurred in non-coding potential regulatory regions. The frequencies of each SNP in EA and AA are presented in Fig. 1.

Haplotypes were estimated by applying the E–M algorithm to compound genotypes. Five haplotypes account for 96% of all variation among EA and seven account for 93% of the variation among AA (Fig. 1). These major haplotypes were found at appreciable frequencies (3–22%) in both racial groups, emphasizing the high level of linkage disequilibrium across the region. For example, haplotype H7 occurred at a frequency of 19.2% in EA, while its expected frequency, based upon individual SNP allele frequencies, was 0.1%. Two haplotypes, H6 and H7, contained multiple unique alleles (e.g., H6 included –2578G and –3306G alleles, while H7 retained –2136T, 767G, and –1385A) at nearly identical frequencies (Fig. 1); therefore, these SNP were nearly in absolute disequilibrium. For the H6 haplotype, linkage disequilibrium extended 14 186 bp and for H7 the linkage disequilibrium extended 31 215 bp.

To discover any genetic influences on susceptibility to HIV-1 infection, frequencies of the *MCP-1–MCP-3–eotaxin* alleles, genotypes, and haplotypes were compared between 824 HIV-1-infected individuals and 247 HREU. Genotypes carrying three specific SNP (–2136T, 767G, –1385A) and their associated H7 haplotype were significantly higher in frequency among the HREU individuals than among seroconverters for all EA [odds ratio (OR), 0.59–0.63; $P = 0.005–0.01$; Table 1] and for EA enrolled in MACS (OR, 0.53; $P < 0.01$; Supplement 1). A similar protective trend was also seen in MHCS (OR, 0.64; $P = 0.18$; Supplement 1) and AA (OR, 0.41; $P = 0.12$) (Table 1), emphasizing the general nature of this effect.

Two additional significant associations were observed with infection among EA; however, each was found only in a single cohort. The 903T genotype frequencies were significantly elevated among MACS seroconverters (OR, 1.99; $P = 0.008$; Supplement 1), and there was a significant excess of genotypes and haplotype H3 carrying the 23Thr allele in MHCS seroconverters (OR, 2.25; $P = 0.03$; Supplement 1).

Survival analyses assessed the rate of progression to

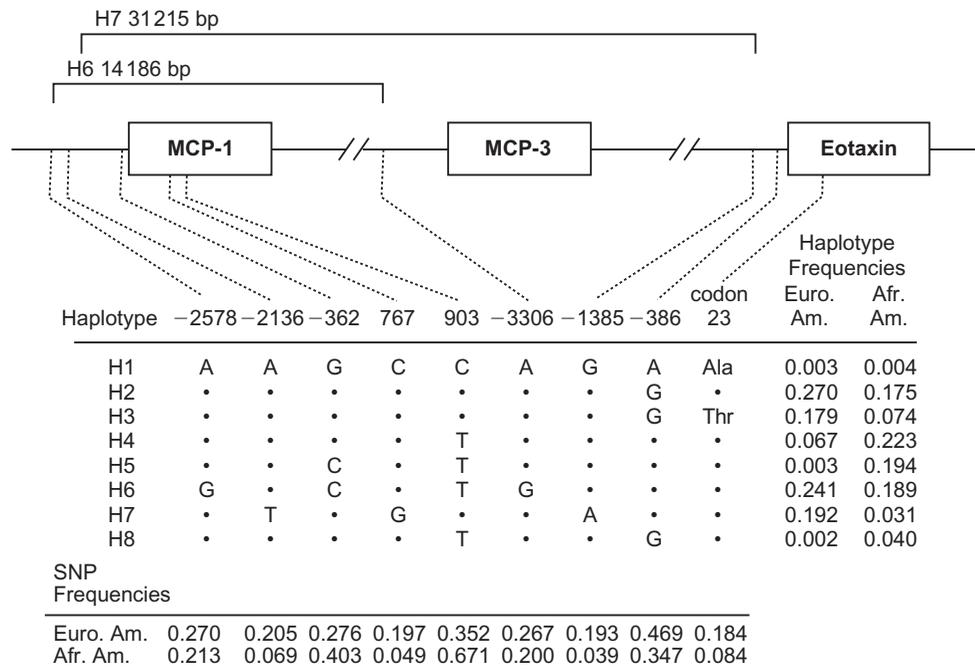


Fig. 1. Molecular map showing positions of the nine single nucleotide polymorphisms (SNP) genotyped in the MCP-1–MCP-3–eotaxin gene cluster on chromosome 17. SNP and haplotype frequencies are indicated for European Americans (Euro. Am.) and African-Americans (Afr. Am.). The common variants in the former are shown in haplotype H1. The lengths (bp) for haplotypes H6 and H7, which contain SNP in near absolute disequilibrium, are presented.

Table 1. Dominant genotype and haplotype frequencies, and comparisons between HIV-1-positive seroconverters and high-risk exposed uninfected individuals.^a

	Frequencies			
	SC	HREU	Odds ratio (95% CI)	P value
European-Americans				
No. individuals examined	600	159		
Single nucleotide polymorphism genotype				
-2136T	0.346	0.456	0.63 (0.44–0.90)	0.01
767G	0.332	0.443	0.62 (0.44–0.89)	0.009
-1385A	0.323	0.443	0.60 (0.42–0.86)	0.005
Haplotype				
H7	0.311	0.433	0.59 (0.41–0.85)	0.005
African-Americans				
No. individuals examined	225	88		
Single nucleotide polymorphism genotype				
-2136T	0.099	0.136	0.69 (0.33–1.47)	0.34
767G	0.063	0.080	0.77 (0.30–1.98)	0.59
-1385A	0.056	0.097	0.55 (0.21–1.46)	0.23
Haplotype				
H7	0.042	0.095	0.41 (0.14–1.25)	0.12

SC, HIV-1-positive seroconverters; HREU, high-risk exposed uninfected individuals; CI, confidence interval.

^aIndividuals homozygous for the CCR5-Δ32 allele were excluded.

AIDS among HIV-1-infected participants with respect to each variant allele, genotype, and haplotype. A total of 600 EA and 225 AA seroconverters were examined using Kaplan–Meier survival analysis and Cox proportional hazards regression. Among AA, increased rates of

progression to AIDS-1987 [relative hazard (RH), 2.18; $P = 0.008$] and to death (RH, 2.52; $P = 0.03$) were associated with -386G, and to AIDS-1987 with haplotype H2 (RH, 2.18; $P = 0.009$), which carries only -386G (Supplements 2–3). In addition, the 903T

SNP was significantly associated with delayed progression to the endpoints CD4 cell count of $< 200 \times 10^6$ cells/l ($P = 0.02$) and death ($P = 0.002$). Since 903T occurs on haplotypes complementary to those containing –386G, these two SNP may be tracking the same effect. Among EA, no significant associations with rates of progression were discovered for any variant allele or haplotype (Supplement 3).

Discussion

The present study provides compelling genetic epidemiological evidence for a role for the cluster encoding MCP-1, MCP-3, and eotaxin chemokines in influencing HIV-1 infection. A significant protective association was found for the –2136T, 767G, and –1385A variants and their haplotype H7 among EA HREU, with a similar trend observed in AA. Differences between racial groups may result from variation in allele frequencies, haplotype structure, cohort composition, or sample sizes. The –2136T and –1385A alleles reside upstream of translation initiation sites where transcription factors are known to bind, and the –1385A SNP modifies the binding site for consensus activator protein-2) [20], although functional studies on transcription are lacking. However, since haplotype H7 extends over 30 kb and contains three genes known to influence HIV-1 pathogenesis and immune regulation, definitive identification of a causal mutation(s) will be challenging.

Since the ligands encoded by these genes do not bind the primary HIV-1 coreceptors CCR5 and CXCR4, the variant alleles contained within H7 presumably act by activating the immune system in response to HIV-1 infection, rather than by blocking viral entry. Therefore, resistant individuals were likely exposed to HIV-1 and were able to control replication and clear virus before the establishment of a productive infection. Although a variety of molecular mechanisms could explain the results, apparent resistance in other high-risk populations has been attributable to an effective anti-HIV cytotoxic T cell response [31–33], HIV-1-specific IgA [34–36], and higher levels of CC chemokines [37]. MCP-1, MCP-3 and eotaxin have a broad range of biological functions and recruit monocytes, eosinophils, and dendritic cells to sites of inflammation. This increased pool of antigen-presenting cells may have beneficial effects in stemming HIV-1 infection in more than one way. While it would seem likely that factors which decrease risk for HIV-1 infection would also decrease risk for progression, the three variant alleles found on haplotype H7 were not shown to modify HIV-1 progression in EA. It may be that the mechanisms required to stem primary, localized HIV-1 replication become less efficient once HIV-1 has become established in multiple cellular compartments. Our results point to a strong role

for the chemokines MCP-1, MCP-3 and/or eotaxin in the early events leading to successful viral clearance. The mechanism for the protective influence of this cluster remains to be elucidated.

Four additional associations (903T, 23Thr, –386G, H2) with transmission and modified progression to AIDS are also reported. Since these influences were each found in only one cohort, they may represent stochastic variation or reflect differences in allele frequencies and haplotype structure between the racial groups or populations. Critical tests will be whether or not these results are confirmed in other cohorts.

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*Electronic database information: Accession numbers and URL for data in this article are as follows: GenBank, <http://www.ncbi.nlm.nih.gov/Genbank/index.html> [for *Homo sapiens* MCP-3 promoter enhancer (X95070), for *H. sapiens* chromosome 17, clone hRPK.215_E_13, complete sequence (AC005549)].*

Supplements

Supplement 1 Additional dominant genotype and haplotype frequencies

Table 2 shows the SNP and haplotypes not included in Table 1 for dominant genotype and haplotype frequencies, and comparisons between SC and HREU.

Supplement 2

Table 3 gives the survival analyses for progression to three AIDS outcomes for MCP-1–MCP-3–eotaxin genotypes and haplotypes under a dominant genetic model among seroconverters.

Table 2. Single nucleotide polymorphisms (SNP) and haplotypes not included in Table 1 for dominant genotype and haplotype frequencies, and comparisons between HIV-1 positive seroconverters (SC) and high-risk exposed uninfected individuals (HREU).

	Frequency		Odds ratio (95% CI)	P value
	SC	HREU		
All European-Americans				
No. individuals examined	600	159		
SNP				
-2578G	0.465	0.415	1.25 (0.86–1.75)	0.26
-362C	0.479	0.430	1.22 (0.86–1.73)	0.28
903T	0.588	0.503	1.41 (0.99–2.00)	0.06
-3306G	0.531	0.576	1.20 (0.84–1.71)	0.31
-386G	0.734	0.715	1.01 (0.75–1.63)	0.63
23Thr	0.350	0.304	1.23 (0.84–1.80)	0.28
Haplotype				
H2	0.472	0.446	1.11 (0.77–1.58)	0.58
H3	0.342	0.299	1.24 (0.84–1.83)	0.28
H4	0.140	0.108	1.32 (0.75–2.30)	0.34
H6	0.434	0.395	1.17 (0.81–1.68)	0.41
All African-Americans				
No. individuals examined	224	88		
SNP				
-2578G	0.371	0.364	1.03 (0.62–1.72)	0.91
-362C	0.655	0.678	0.90 (0.53–1.53)	0.70
903T	0.876	0.862	1.13 (0.55–2.34)	0.74
-3306G	0.367	0.368	0.99 (0.60–1.67)	1.00
-386G	0.544	0.574	0.88 (0.53–1.4)	0.63
23Thr	0.161	0.159	1.02 (0.51–2.03)	0.97
Haplotype				
H2	0.298	0.318	0.93 (0.50–1.73)	0.82
H3	0.141	0.127	1.11 (0.47–2.60)	0.82
H4	0.382	0.413	0.87 (0.48–1.58)	0.66
H5	0.351	0.349	0.97 (0.53–1.79)	0.93
H6	0.356	0.286	1.47 (0.77–2.80)	0.24
H8	0.110	0.064	2.27 (0.58–8.88)	0.24
Multicenter AIDS Cohort Study				
No. individuals examined	342	74		
SNP				
-2578G	0.480	0.365	1.60 (0.96–2.69)	0.07
-2136T	0.321	0.473	0.53 (0.32–0.88)	0.01
-362C	0.500	0.365	1.74 (1.04–2.90)	0.04
767G	0.305	0.460	0.52 (0.31–0.86)	0.01
903T	0.615	0.446	1.99 (1.20–3.30)	0.008
-3306G	0.513	0.630	1.62 (0.96–2.72)	0.07
-1385A	0.304	0.446	0.54 (0.32–0.91)	0.02
-386G	0.737	0.757	0.90 (0.50–1.62)	0.73
23Thr	0.318	0.365	0.81 (0.48–1.38)	0.44
Haplotype				
H2	0.498	0.452	1.20 (0.72–2.01)	0.49
H3	0.301	0.356	0.78 (0.45–1.35)	0.38
H4	0.155	0.110	1.51 (0.68–3.36)	0.31
H6	0.463	0.343	1.66 (0.97–2.84)	0.06
H7	0.288	0.438	0.52 (0.31–0.88)	0.01
Multicenter Hemophilia Cohort				
No. individuals examined	170	50		
SNP				
-2578G	0.462	0.400	1.29 (0.68–2.44)	0.44
-2136T	0.329	0.449	0.60 (0.31–1.15)	0.13
-362C	0.468	0.429	1.17 (0.62–2.22)	0.63
767G	0.324	0.429	0.64 (0.33–1.22)	0.17
903T	0.539	0.520	1.08 (0.57–2.03)	0.81
-3306G	0.465	0.420	1.20 (0.64–2.27)	0.57
-1385A	0.324	0.429	0.64 (0.33–1.22)	0.18
-386G	0.744	0.694	1.28 (0.64–2.59)	0.49
23Thr	0.421	0.245	2.25 (1.09–4.62)	0.03
Haplotype				
H2	0.463	0.490	0.90 (0.47–1.71)	0.74
H3	0.414	0.245	2.18 (1.06–4.49)	0.04
H4	0.093	0.122	0.73 (0.29–2.07)	0.55
H6	0.414	0.408	1.02 (0.53–1.96)	0.95
H7	0.321	0.429	0.63 (0.34–1.21)	0.17

CI, confidence intervals.

Individuals homozygous for the *CCR5* Δ32 allele were excluded.

Table 3. Survival analyses by Cox regression of progression to three AIDS outcomes for MCP-1–MCP-3–eotaxin genotypes and haplotypes under a dominant genetic model among seroconverters adjusted for other known AIDS-modifying factors (see Methods).

	CD4 cell count < 200 × 10 ⁶ cells/l		AIDS-1987 ^a		Death ^b	
	RH	P value	RH	P value	RH	P value
European Americans (n = 600)						
SNP						
–2578G	0.94	0.55	0.97	0.77	1.10	0.47
–2136T	0.88	0.26	1.00	0.99	0.92	0.56
–362C	0.99	0.89	1.01	0.90	1.17	0.24
767G	0.92	0.47	1.01	0.95	0.92	0.54
903T	0.89	0.34	0.91	0.47	1.00	0.98
–3306G	0.95	0.63	1.01	0.97	1.15	0.27
–1385A	0.91	0.45	1.02	0.88	1.01	0.95
–386G	1.06	0.68	1.03	0.82	1.06	0.69
23Thr	1.05	0.67	1.04	0.74	1.13	0.37
Haplotype						
H2	1.09	0.50	1.05	0.70	0.99	0.94
H3	1.09	0.53	1.08	0.58	1.09	0.59
H4	0.87	0.46	1.01	0.94	0.96	0.84
H6	0.94	0.65	0.92	0.54	1.02	0.88
H7	0.98	0.84	1.09	0.53	1.06	0.70
African-Americans (n = 225)						
SNP						
–2578G	0.89	0.62	0.87	0.61	0.92	0.82
–2136T	1.94	0.06	1.08	0.88	1.04	0.96
–362C	0.56	0.01	0.63	0.08	0.55	0.10
767G	1.11	0.82	0.75	0.69	0.66	0.69
903T	0.53	0.02	0.61	0.14	0.29	0.003
–3306G	0.90	0.66	0.99	0.97	1.11	0.78
–1385A	1.56	0.37	1.00	1.00	0.74	0.77
–386G	1.26	0.32	2.18	0.008	2.52	0.03
23Thr	1.20	0.52	1.40	0.32	2.86	0.01
Haplotype						
H2	1.21	0.49	2.18	0.009	1.88	0.13
H3	1.29	0.43	1.22	0.60	2.64	0.04
H4	1.12	0.67	0.74	0.34	0.22	0.14
H5	0.68	0.18	0.48	0.04	0.42	0.10
H6	0.91	0.69	1.00	0.99	1.01	0.98
H7	1.83	0.35	0.72	0.75	1.41	0.75
H8	1.32	0.54	1.87	0.23	0.23	0.28

RH, relative hazards.

^aAIDS-1987 as defined by the Centers for Disease Control and Prevention [22].

^bAIDS-related death.

Supplement 3 Progression to death for different modelled genotypes

Figure 2 shows Kaplan–Meier curves for progression to death and AIDS-1987 for MCP-1 903T and eotaxin 386G genotypes, respectively, under the dominant genetic model.

References

- O'Brien SJ, Nelson GW, Winkler CA, Smith MW. Polygenic and multifactorial disease gene association in man: lessons from AIDS. *Annu Rev Genet* 2000, **34**:563–591.
- Baggiolini M, Dewald B, Moser B. Interleukin-8 and related chemotactic cytokines: CXC and CC chemokines. *Adv Immunol* 1994, **55**:97–179.
- Sallusto F, Schaerli P, Loetscher P, Schaniel C, Lenig D, Mackay CR, et al. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur J Immunol* 1998, **28**:2760–2769.
- Sozzani S, Allavena P, D'Amico G, Luini W, Bianchi G, Kataura M, et al. Differential regulation of chemokine receptors during dendritic cell maturation: a model for their trafficking properties. *J Immunol* 1998, **161**:1083–1086.
- Lin CL, Suri RM, Rahdon RA, Austyn JM, Roake JA. Dendritic cell chemotaxis and transendothelial migration are induced by distinct chemokines and are regulated on maturation. *Eur J Immunol* 1998, **28**:4114–122.
- Swingler S, Mann A, Jacque J, Brichacek B, Sasseville VG, Williams K, et al. HIV-1 Nef mediates lymphocyte chemotaxis and activation by infected macrophages. *Nat Med* 1999, **5**:997–1003.
- Schmidtmayerova H, Nottet HS, Nuovo G, Raabe T, Flanagan CR, Dubrovsky L, et al. Human immunodeficiency virus type 1 infection alters chemokine beta peptide expression in human monocytes: implications for recruitment of leukocytes into brain and lymph nodes. *Proc Natl Acad Sci USA* 1996, **93**:700–704.
- Weiss JM, Nath A, Major EO, Berman JW. HIV-1 Tat induces monocyte chemoattractant protein-1-mediated monocyte transmigration across a model of the human blood–brain barrier and

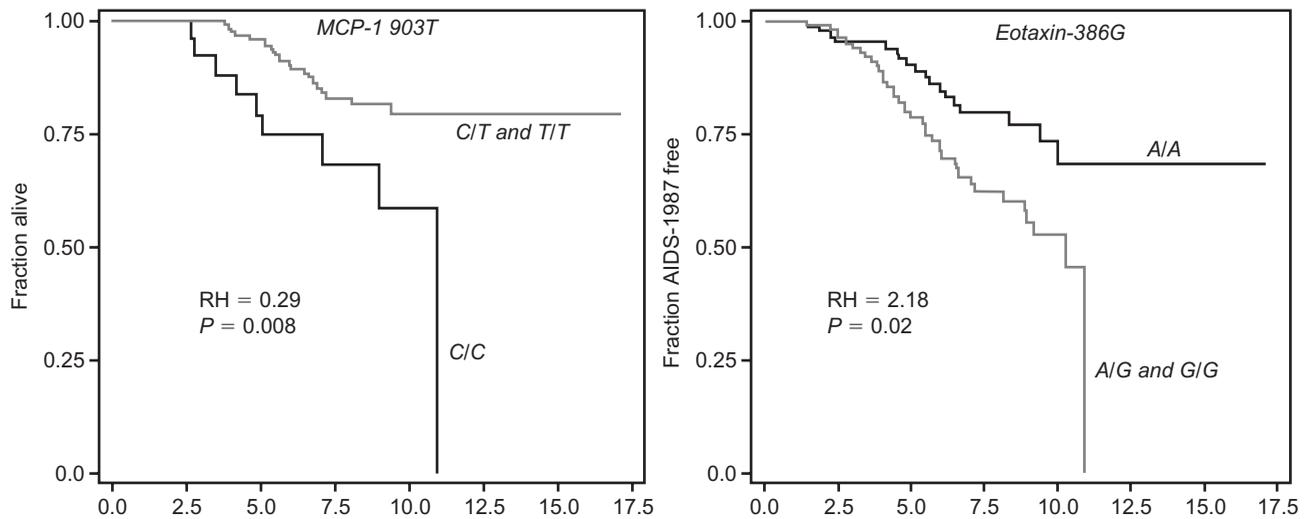


Fig. 2. Kaplan–Meier curves for progression to AIDS outcomes for *MCP-1 903T* and *eotaxin 386G* genotypes in African-Americans under the dominant genetic model. (a) Progression to AIDS-related death for *MCP-1 903T* genotype. (b) Progression to AIDS-1987 (as defined by the Centers for Disease Control and Prevention [22]) for *eotaxin 386G* genotype. RH, Cox regression relative hazard; *P* value, Kaplan–Meier Wilcoxon values.

- up-regulates CCR5 expression on human monocytes. *J Immunol* 1999, **163**:2953–2959.
9. Vicenzi E, Alfano M, Ghezzi S, Gatti A, Veglia F, Lazzarin A, Sozzani S, et al. Divergent regulation of HIV-1 replication in PBMC of infected individuals by CC chemokines: suppression by RANTES, MIP-1alpha, and MCP-3, and enhancement by MCP-1. *J Leukoc Biol* 2000, **68**:405–412.
 10. Greco G, Mackewicz C, Levy JA. Sensitivity of human immunodeficiency virus infection to various alpha, beta and gamma chemokines. *J Gen Virol* 1999, **80**:2369–2373.
 11. Hadida F, Vieillard V, Autran B, Clark-Lewis I, Baggiolini M, Debre P. HIV-specific T cell cytotoxicity mediated by RANTES via the chemokine receptor CCR3. *J Exp Med* 1998, **188**:609–614.
 12. Vlahov D. The ALIVE study, a longitudinal study of HIV-1 infection in intravenous drug users: description of methods and characteristics of participants. *NIDA Res Monogr Ser* 1991, **109**:75–100.
 13. Hilgartner MW, Donfield SM, Willoughby A, Contant CF, Jr, Evatt BL, Gomperts ED, et al. Hemophilia growth and development study. Design, methods, and entry data. *Am J Pediatr Hematol Oncol* 1993, **15**:208–218.
 14. Goedert JJ, Kessler CM, Aledort LM, Biggar RJ, Andes WA, et al. A prospective study of human immunodeficiency virus type 1 infection and the development of AIDS in subjects with hemophilia. *N Engl J Med* 1989, **321**:1141–1148.
 15. Detels R, Liu Z, Hennessey K, Kan J, Visscher BR, Taylor JM, et al. Resistance to HIV-1 infection. Multicenter AIDS Cohort Study. *J Acquir Immune Defic Syndr* 1994, **7**:1263–1269.
 16. Kaslow RA, Ostrow DG, Detels R, Phair JP, Polk BF, Rinaldo CR, Jr. The Multicenter AIDS Cohort Study: rationale, organization, and selected characteristics of the participants. *Am J Epidemiol* 1987, **126**:310–318.
 17. Rutherford GW, Lifson AR, Hessel NA, Darrow WW, O'Malley PM, Buchbinder SP, et al. Course of HIV-1 infection in a cohort of homosexual and bisexual men: an 11 year follow up study. *Br Med J* 1990, **301**:1183–1188.
 18. Salkowitz JR, Purvis SF, Meyerson H, Zimmerman P, O'Brien TR, Aledort L, et al. Characterization of high-risk HIV-1 seronegative hemophiliacs. *Clin Immunol* 2001, **98**:200–211.
 19. Vlahov D, Polk BF. Perspectives on infection with HIV-1 among intravenous drug users. *Psychopharmacol Bull* 1988, **24**:325–329.
 20. Hein H, Schluter C, Kulke R, Christophers E, Schroder JM, Bartels J. Genomic organization, sequence, and transcriptional regulation of the human eotaxin gene. *Biochem Biophys Res Commun* 1997, **237**:537–542.
 21. Modi WS, Bergeron J, Sanford M. The human MIP-1beta chemokine is encoded by two paralogous genes, *ACT-2* and *LAG-1*. *Immunogenetics* 2001, **53**:543–549.
 22. Centers for Disease Control. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. *MMWR* 1987, **36**(suppl 1):1–155.
 23. Carrington M, Nelson GW, Martin MP, Kissner T, Vlahov D, Goedert JJ, et al. HLA and HIV-1: heterozygote advantage and B*35–Cw*04 disadvantage. *Science* 1999, **283**:1748–1752.
 24. Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CCR5 structural gene. *Science* 1996, **273**:1856–1862.
 25. Gao X, Nelson GW, Karacki P, Martin MP, Phair J, Kaslow R, et al. Effect of a single amino acid change in MHC class I molecules on the rate of progression to AIDS. *N Engl J Med* 2001, **344**:1668–1675.
 26. Smith MW, Dean M, Carrington M, Winkler C, Huttley GA, Lomb D, et al. Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. *Science* 1997, **277**:959–965.
 27. Winkler C, Modi W, Smith MW, Nelson GW, Wu X, Carrington M, et al. Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant. *Science* 1998, **279**:389–393.
 28. Long JC, Williams RC, Urbanek M. An E–M algorithm and testing strategy for multiple-locus haplotypes. *Am J Hum Genet* 1995, **56**:799–810.
 29. Rovin BH, Lu L, Saxena R. A novel polymorphism in the MCP-1 gene regulatory region that influences MCP-1 expression. *Biochem Biophys Res Commun* 1999, **259**:344–348.
 30. Bartels J, Schluter C, Richter E, Noso N, Kulke R, Christophers E, et al. Human dermal fibroblasts express eotaxin: molecular cloning, mRNA expression, and identification of eotaxin sequence variants. *Biochem Biophys Res Commun* 1996, **225**:1045–1051.
 31. Rowland-Jones S, Sutton J, Ariyoshi K, Dong T, Gotch F, McAdam S, et al. HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. *Nat Med* 1995, **1**:59–64.
 32. Goh WC, Markee J, Akridge RE, Meldorf M, Musey L, Karchmer T, et al. Protection against human immunodeficiency virus type

- 1 infection in persons with repeated exposure: evidence for T cell immunity in the absence of inherited CCR5 coreceptor defects.** *J Infect Dis* 1999, **179**:548–557.
33. Bernard NF, Yannakis CM, Lee JS, Tsoukas CM. **Human immunodeficiency virus (HIV)-specific cytotoxic T lymphocyte activity in HIV-exposed seronegative persons.** *J Infect Dis* 1999, **179**:538–547.
34. Mazzoli S, Lopalco L, Salvi A, Trabattoni D, Lo Caputo S, Semplici F, *et al.* **Human immunodeficiency virus (HIV)-specific IgA and HIV neutralizing activity in the serum of exposed seronegative partners of HIV-seropositive persons.** *J Infect Dis* 1999, **180**:871–875.
35. Kaul R, Trabattoni D, Bwayo JJ, Arienti D, Zagliani A, Mwangi FM, *et al.* **HIV-1-specific mucosal IgA in a cohort of HIV-1-resistant Kenyan sex workers.** *AIDS* 1999, **13**:23–29.
36. Belec L, Ghys PD, Hocini H, Nkengasong JN, Tranchot-Diallo J, Diallo MO, *et al.* **Cervicovaginal secretory antibodies to human immunodeficiency virus type 1 (HIV-1) that block viral transcytosis through tight epithelial barriers in highly exposed HIV-1-seronegative African women.** *J Infect Dis* 2001, **184**:1412–1422.
37. Shieh B, Yan YP, Ko NY, Liao YE, Liu YC, Lin HH, *et al.* **Detection of elevated serum beta-chemokine levels in seronegative Chinese individuals exposed to human immunodeficiency virus type 1.** *Clin Infect Dis* 2001, **33**:273–279.