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Novel Alleles of the Chemokine-Receptor Gene *CCR5*

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Summary

The *CCR5* gene encodes a cell-surface chemokine-receptor molecule that serves as a coreceptor for macrophage-tropic strains of HIV-1. Mutations in this gene may alter expression or function of the protein product, thereby altering chemokine binding/signaling or HIV-1 infection of cells that normally express CCR5 protein. Indeed, homozygotes for a 32-bp deletion allele of *CCR5* (*CCR5*-Δ*32*), which causes a frameshift at amino acid 185, are relatively resistant to HIV-1 infection. Here we report the identification of 16 additional mutations in the coding region of the *CCR5* gene, all but 3 of which are codon altering or "nonsynonymous." Most mutations were rare (found only once or twice in the sample); five were detected exclusively among African Americans, whereas eight were observed only in Caucasians. The mutations included 11 codon-altering nonsynonymous variants, one trinucleotide deletion, one chain-termination mutant, and three synonymous mutations. The high predominance of codon-altering alleles among *CCR5* mutants (14/17 [81%], including *CCR5*-Δ*32*) is consistent with an adaptive accumulation of function-altering alleles for this gene, perhaps as a consequence of historic selective pressures.

Introduction

The *CCR5* gene encodes a cell-surface receptor that binds the β-chemokines RANTES, MIP-1α, and MIP-1β (Samson et al. 1996*a*), causing migration of the receptor-bearing cell toward an increasing concentration of the chemokine. This chemotactic response results in recruitment of leukocytes to sites of inflammation (Murphy 1996; Premach and Schall 1996). A second class of ligands for CCR5 were recently shown to be the envelope glycoproteins of macrophage-tropic (M-tropic) isolates of HIV-1 (Alkhatib et al. 1996; Choe et al. 1996; Deng et al. 1996; Doranz et al. 1996; Dragic et al. 1996). These isolates infect macrophages and primary T cells and are present early after seroconversion, during the asymptomatic period (Roos et al. 1992; Schuitemaker et al. 1992; Connor and Ho 1994), indicating a role for M-tropic isolates in initiation of HIV-1 infection. However, sometimes infection can occur in the absence of CCR5 (Biti et al. 1997; O'Brien et al. 1997; Theodorou et al. 1997), suggesting that T-tropic viruses (those which infect primary T cells and T cell lines) may also initiate HIV-1 infection in rare cases. Identification of an allele characterized by a 32-bp deletion in the coding region of the *CCR5* gene, *CCR5*-Δ*32*, has been shown to confer near-complete protection against HIV-1 infection in individuals homozygous for the mutant allele (Dean et al. 1996; Liu et al. 1996; Samson et al. 1996*b*). Although individuals homozygous for the *CCR5*-Δ*32* allele fail to express a detectable CCR5 receptor on lymphoid cell surfaces, they display no clinical symptoms and appear to be immunologically healthy. Since other genetically homologous chemokine receptors bind an overlapping set of ligands, it is possible that chemokine-receptor functional redundancy can compensate for *CCR5* absence in homozygous *CCR5*-Δ*32* individuals (Premack and Schall 1996).

On the basis of the geographic distribution of the *CCR5*-Δ*32* allele, as well as on the intrahaplotypic variation determined by using flanking microsatellite loci in strong linkage disequilibrium with *CCR5*-Δ*32*, we have estimated that the 32-bp deletion occurred on the order of 4,000 years ago (J. C. Stephens, personal communication). Since that time, the allele has increased to a frequency of as high as 13%, and perhaps higher, in northern Europeans, but it is lacking in Africans and Asians (Huang et al. 1996; Samson et al. 1996*b*; Martinson et al. 1997). The rapid increase in frequency of this mutant allele during a relatively short period of time suggests that selection favoring the *CCR5*-Δ*32* allele may have occurred (and, perhaps, is still occurring) in certain populations. Had historic or ongoing selective pressures on *CCR5*-Δ*32* been operative, then other mu-

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tations in the *CCR5* gene could have been objects of the same selective pressures. Indeed, seven additional alleles of the *CCR5* gene have been identified recently in four ethnic groups, although none of these alleles were observed in the 50 Caucasians sampled (Ansari-Lari et al. 1997).

Recent studies have attempted to identify regions of the CCR5 molecule that are important in membrane fusion of HIV-1, and the resulting data indicate an interaction, between virus and receptor, requiring multiple sites of binding (Atchison et al. 1996; Rucker et al. 1996). However, cellular signaling through the CCR5 receptor is not required for HIV-1 fusion, and deletion of the intracellular carboxy-terminal portion of CCR5 does not affect the ability of HIV-1 to infect cells in vitro. Therefore, amino acid alterations in the extracellular domains of CCR5 are the best candidates for inhibiting (or enhancing) HIV-1 binding and fusion. Initial screening of HIV-1–exposed populations for mutations in *CCR5* suggests that, except for *CCR5-Δ32,* alleles of this gene exist at a very low frequency (Dean et al. 1996). Nevertheless, identification of these alleles may provide information regarding functionally significant residues/segments of the CCR5 molecule and allow predictions of allelic selection. Here we report the identification of 16 alleles additional to *CCR5-Δ32,* only 3 of which were silent substitutions.

Material and Methods

DNA Samples

DNA samples were obtained from individuals participating in one of five AIDS cohorts, which have been described in a previous report (Dean et al. 1996).

PCR Amplification and SSCP

Variations in the nucleotide sequence of the *CCR5* gene were analyzed by SSCP analysis of the following PCR products: (1) the entire coding region of the *CCR5* gene, amplified with primers F2 (5′-GGTGGAACAAGATGGATTAT) and R2 (5′-CATGTGCACAACTCTGACTG), followed by *Hin*fI digestion, or (2) four overlapping segments of the gene, encompassing the entire coding region, amplified with primer pairs F2 and R3 (5′-GCCCTGTCAAGAGTTGACAC), F2a (5′-ATGCTGCCGCCCAGTGGGAC) and R2a (5′-GTATGGAAAATGAGAGCTGC), F3a (5′-GAAGGTCTTCATTACACCTG) and R3a (5′-AGAATTCCTGGAAGGTGTTC), and F5 (5′-TCTCTTCTGGGCTCCCTACA) and R5a (5′-CCAGCCCACTTGAGTCCGTG). PCR amplifications and subsequent SSCP analysis were performed as reported previously (Cullen et al. 1997).

Sequencing

Direct sequencing of PCR products was performed by isolation of the product from an agarose gel, followed by sequencing with Dye Terminators (Perkin-Elmer). Sequencing products were resolved on an ABI 373 automated sequencer, and all alleles were sequenced in both directions.

Results

Identification and Ethnic Origins of CCR5 *Mutations*

Screening ~700 Caucasians and ~700 African Americans for variation in the coding region of the *CCR5* gene resulted in the identification of 11 novel point mutations, a 3-bp deletion, and four previously reported point mutations (Ansari-Lari et al. 1997) (fig. 1 and table 1). Twelve of the 15 point mutations were nonsynonymous changes resulting in amino acid alterations, and three were synonymous. Eleven point mutations represent transversions, and four represent transitions. All alleles of *CCR5* that are shown in table 1 are found at low frequencies relative to the wild-type and *CCR5-Δ32* alleles. Eight of the mutations were identified only in Caucasians, five were found exclusively among African Americans, one was found only in a Hispanic, and two mutations, L55Q and A335V, were present in both Caucasians and African Americans. The higher frequency of the L55Q mutation in Caucasians (.041) compared with that in African Americans (.006) suggests that the allele was added to the pool of *CCR5* polymorphisms in African Americans because of recent admixture. Alternatively, the A335V mutation is found at a higher frequency in African Americans than in Caucasians and therefore may either be older than the split between the two groups or be of recent African origin with admixture to Caucasians.

Location and Conservation of Mutations

An alignment of the human CCR5 amino acid sequence with other chemokine-receptor sequences is shown in figure 1*A*. Seven of the mutations (C20S, L55Q, A73V, C101X, R223Q, 228ΔK, and G301V) have occurred at positions that are highly conserved throughout β-chemokine receptors; of these seven, three (C20S, C101X, and G301V) are conserved in the α-chemokine receptor, CXCR4, as well.

Mutations in the *CCR5* gene resulted in amino acid alterations throughout the molecule, with a slight concentration near the N-terminus (fig. 1*B*). Furthermore, mutations were observed in regions of the gene encoding transmembrane, intracellular, and extracellular domains of the molecule. Since the conserved cysteine at position 20 is proposed to form a disulfide bond with cysteine

A

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           1           L        S         S             F             Q     S             V            86
  CCR5     MDYQVS SPIYDINYYT SEPCQKINVK QIAARLLPPL YSLVFIFGFV GNMLVILILI NCCRLKSMTD IYLLNLAISD LFFLLTVPFW
 CCR2B MLSTSRSRFI RNTNESGEEV TTFFDYDY.. GAPCHKFDVK QIGAQLLPPL YSLVFIFGFV GNMLVVLILI NCKKLKCLTD IYLLNLAISD LLFLITLPLW
  CCR1          M ETP.NTTEDY DTTTEFDYGD ATPCQKVNER AFGAQLLPPL YSLVFVIGLV GNILVVLVLV QYKRLKNMTS IYLLNLAISD LLFLFTLPFW
  CCR3          M TTSLDTVETF GTTSYYD.DV GLLCEKADTR ALMAQFVPPL YSLVFTVGLL GNVVVVMILI KYRRLRIMTN IYLLNLAISD LLFLVTLPFW
  CCR4     MNPTDI ADTTLDESIY SNYYLYE.SI PKPCTKEGIK AFGELFLPPL YSLVFVFGLL GNSVVVLVLF KYKRLRSMTD VYLLNLAISD LLFVFSLPFW
 CXCR4     MEGISI YTSDNYTEEM GSG.DYD.SM KEPCFREENA NFNKIFLPTI YSIIFLTGIV GNGLVILVMG YQKKLRSMTD KYRLHLSVAD LLFVITLPFW

       87          X                                                                              185
  CCR5 AHYA.AAQWD FGNTMCQLLT GLYFIGFFSG IFFIILLTID RYLAVVHAVF ALKARTVTFG VVTSVITWVV AVFASLPGII FTRSQKEGLH YTCSSHFPYS
 CCR2B AHSA.ANEWV FGNAMCKLFT GLYHIGYFGG IFFIILLTID RYLAIVHAVF ALKARTVTFG VVTSVITWLV AVFASVPGII FTKCQKEDSV YVCGPYFPRG
  CCR1 IDYKLKDDWV FGDAMCKILS GFYYTGLYSE IFFIILLTID RYLAIVHAVF ALRARTVTFG VITSIIIWAL AILASMPGLY FSKTQWEFTH HTCSLHFPHE
  CCR3 IHYVRGHNWV FGHGMCNLLS GFYHTGLYSE IFFIILLTID RYLAIVHAVF ALRARTVTFG VITSIVTWGL AVLAALPEFI FYETEELFEE TLCSALYPED
  CCR4 GYYA.ADQWV FGLGLCKMIS WMYLVGFYSG IFFVMLMSID RYLAIVHAVF SLRARTLTYG VITSLATWSV AVFASLPGFL FSTCYTERNH TYCKTKYSLN
 CXCR4 AVDAVAN.WY FGNFLCKAVH VIYTVNLYSS VLILAFISLD RYLAIVHATN SQRPRKLLAE KVVYVGVWIP ALLLTIPDFI FANVSEADDR YICDRFYPND

       186                                     Q     Δ                                                284
  CCR5 QYQFWKNFQT LKIVILGLVL PLLVMVICYS GILKTLLRCR NEKKRHRAVR LIFTIMIVYF LFWAPYNIVL LLNTFQEF.F GLNNCSSSNR LDQAMQVTET
 CCR2B ....WNNFHT IMRNILGLVL PLLIMVICYS GILKTLLRCR NEKKRHRAVR VIFTIMIVYF LFWTPYNIVI LLNTFQEF.F GLSNCESTSQ LDQATQVTET
  CCR1 SLREWKLFQA LKLNLFGLVL PLLVMIICYT GIIKILLRRP NEKK.SKAVR LIFVIMIIFF LFWTPYNLTI LISVFQDF.L FTHECEQSRH LDLAVQVTEV
  CCR3 TVYSWRHFHT LRMTIFCLVL PLLVMAICYT GIIKTLLRCP SKKK.YKAIR LIFVIMAVFF IFWTPYNVAI LLSSYQSI.L FGNDCERSKH LDRVMLVTEV
  CCR4 ST.TWKVLSS LEINILGLVI PLGIMLFCYS MIIRTLQHCK NEKK.NKAVK MIFAVVVLFL GFWTPYNIVL FLETLVEL.E VLQDCTFERY LDYAIQATET
 CXCR4 ...LWVVVFQ FQHIMVGLIL PGIVILSCYC IIISKLSHSK GHQKR.KALK TTVILILAFF ACWLPYYIGI SIDSFILLEI IKQGCEFENT VHKWISITEA

       285            V                                      V   F            332
  CCR5 LGMTHCCINP IIYAFVGEKF RNYLLVFFQK .HIAKRFCKC CSIFQQEAPE RASSVYTRST GEQEISVGL
 CCR2B LGMTHCCINP IIYAFVGEKF RRYLSVFFRK .HITKRFCKQ CPVFYRETVD GVTSTNTPST GEQEVSAGL
  CCR1 IAYTHCCVNP VIYAFVGERF RKYLRQLFHR .RVAVHLVKW LPFLSVDRLE RVSST.SPST GEHELSAGF
  CCR3 IAYSHCCMNP VIYAFVGERF RKYLRHFFHR .HLLMHLGRY IPFLPSEKLE RTSSV.SPST AEPELSIVF
  CCR4 LAFVHCCLNP IIYFFLGEKF RKYILQLFKT CRGLFVLCQY CGLLQIYSAD TPSSSYTQST MDHDLHDAL
 CXCR4 LAFFHCCLNP ILYAFLGAKF KTSAQHALTS VSRGSSL... .KILSKGKRG GHSSVSTESE SSSFHSS
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B

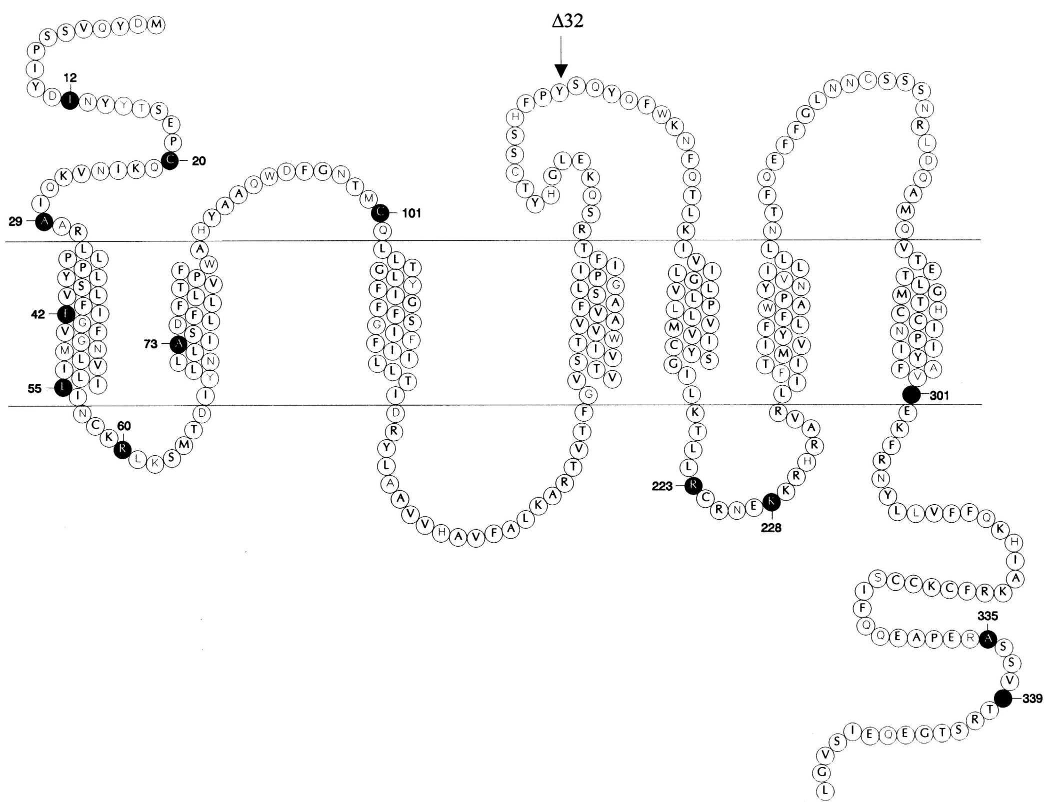

Figure 1 Amino acid sequence variation in the CCR5 protein. *A,* Sequence alignment of five β-chemokine receptors and the α-chemokine receptor, CXCR4. Positions in the CCR5 protein having altered amino acids, as deduced from the nucleotide sequence, are shown above the wild-type *CCR5* sequence. A dot (.) denotes the absence of an amino acid, and underlining denotes segments that are proposed transmembrane regions of the molecule. *B,* Diagram of the CCR5 molecule spanning the membrane. Positions where alterations were identified have been blackened. The arrow indicates the beginning of the region affected by the Δ32 mutation.

Table 1

Genetic Variants of the *CCR5* Gene

Variant[a]	Nucleic Acid Substitution	No. of Alelles Observed/Total No. of Chromosomes (Frequency)[b]	
		Caucasians	African Americans
I12L	A25C	1/382 (.003)	0/664 (.0)
C20S	T58A	2/698 (.003)	0/664 (.0)
A29S	G85T	NT	1/64 (.015)
I42F	A124T	1/170 (.001)	NT
L55Q	T164A	29/708 (.041)	5/664 (.007)
R60S	G180T	NT	1/76 (.013)
A73V	C218T	3/462 (.002)	0/664 (.0)
S75S	T215C[c]	0/212 (.0)	9/664 (.013)
C101X	T303A	NT	1/70 (.014)
I164I	C492A[c]	1/98 (.010)	NT
Δ32(185)	Δ32	520/5,210 (.10)	38/2,030 (.019)
R223Q	G668A	1/64 (.016)	NT
228delK	680del3	1/490 (.002)	0/494 (.0)
V300V[c,d]	C900A	1/242 (.0)	0/100 (.0)
G301V	G902T	1/90 (.011)	0/268 (.0)
A335V	C1004T	1/174 (.006)	12/484 (.025)
Y339F	A1016T	0/242 (.0)	3/116 (.026)

[a] Except in the case of 228delK, the first letter in each entry denotes the wild-type amino acid; the number denotes the position; and the letter following the number denotes the mutated amino acid; 228delK is a triplet deletion of lysine (K) at position 228. In the case of I12L, A29S, and Y339F, the substitutions are conservative, based on net charge; all other substitutions are nonconservative, resulting in alteration of amino acid charge.

[b] "NT" denotes that controls for the variants were not included on gels representing that particular region; thus, it is not certain that the variant would have been identifiable if it indeed had been present on that gel.

[c] Synonymous, non–codon altering.

[d] Found in a single Hispanic individual.

at position 269 (Combadiere et al. 1996; Samson et al. 1996*a*), the C20S mutation located extracellularly near the N-terminus is very likely to alter ligand binding to CCR5.

Clinical Data in Individuals Having Novel CCR5 *Alleles*

Cohorts of individuals at high risk for HIV-1 infection were chosen for screening the *CCR5* gene for mutations, in order to identify potential alleles (in addition to *CCR5*-Δ*32*) affecting virus infectivity or progression to AIDS. Unlike *CCR5*-Δ*32*, none of the novel alleles were found at a frequency high enough to allow evaluation of a potential role in protection against HIV-1, when a population-survey approach was used. Indeed, no individuals were discovered who were homozygous for any of the novel alleles. There were, however, several individuals heterozygous for a rare mutation and the *CCR5*-Δ*32* allele. Since *CCR5*-Δ*32* has been shown to confer strong resistance to HIV-1 infection among homozygotes, as well as postponement of AIDS progression among *CCR5*-+/Δ*32* heterozygotes (Dean et al. 1996; Huang et al. 1996; Michael et al. 1997; Smith et al. 1997; Zimmerman et al. 1997), we examined the clinical outcomes of individuals with novel mutations, to detect evidence for protection against HIV-1 infection or progression to AIDS or both (table 2).

Four nonsynonymous variants (I12L, I42F, L55Q, and A73V) were found as heterozygotes with *CCR5*-Δ*32*, and patients heterozygous for all but I42F included HIV-1–infected patients. The position-42 isoleucine, which is altered in the I42F, is conserved between CCR5 and CCR2 (fig. 1*A*), as well as among CCR5 in other species. Patients with mutations I12L or A73V heterozygous with *CCR5*-Δ*32* were infected with HIV-1, although the I12L/*CCR5*-Δ*32* heterozygotes have survived nearly 14 years without progressing to AIDS, an observation consistent with possible protection in AIDS progression.

The more common L55Q and A335V mutations occurred in both Caucasian and African American individuals. Of six L55Q/*CCR5*-Δ*32* heterozygotes, four were infected with HIV-1 and two were not. The infected individuals progressed to AIDS in 8.2–14.1 years (table 2), which is not different from the median time to AIDS, 10–12 years, indicating no obvious effect of this mutation on infection or disease progression. There were no A335V/*CCR5*-Δ*32* heterozygotes observed, precluding an analysis of infection restriction by this CCR5-variant molecule. The genotype *CCR5*-+/A335V did not display an obvious effect on progression to AIDS, since three patients progressed to AIDS in 5–7 years, but oth-

Table 2

Clinical Description of Individuals with *CCR5* Alleles Altering Amino Acid Sequences

Variant (Patient)	Risk Group	Race	HIV-1 Status	AIDS Status[a]	No. of Years HIV−1 Positive and AIDS Negative[b]	*CCR5*-Δ*32* Genotype[c]
I12L	Hemophilia	Caucasian	Positive	Negative	13.6	+/*CCR5*-Δ*32*
C20S(1)	Homosexual	Caucasian	Positive	Negative	15.1	+/+
C20S(2)	Homosexual	Caucasian	Positive	Positive	12.2	+/+
A29S	IV drug user	African American	Positive	Negative	5.7	+/+
I42F	Homosexual	Caucasian	Negative	Negative	NA	+/*CCR5*-Δ*32*
L55Q(1)[d]	Hemophilia	African American	Positive	Negative	8	+/+
L55Q(2)	IV drug user	African American	Positive	Negative	7.4	+/+
L55Q(3)	IV drug user	African American	Positive	Negative	8.5	+/+
L55Q(4)	IV drug user	African American	Negative	Negative	NA	+/+
L55Q(5)	IV drug user	African American	Negative	Negative	NA	+/+
R60S	IV drug user	African American	Negative	Negative	NA	+/+
A73V(1)	Hemophilia	Caucasian	Positive	Positive	11.9	+/*CCR5*-Δ*32*
A73V(2)	IV drug user	Caucasian	Negative	Negative	NA	+/+
A73V(3)	Homosexual	Caucasian	Positive	Positive	7.8	+/*CCR5*-Δ*32*
C101X	IV drug user	African American	Positive	Negative	6.3	+/+
R223Q	Homosexual	Caucasian	Positive	Positive	13.1	+/+
228delK	IV drug user	Caucasian	Positive	Negative	11.3	+/+
G301V	Homosexual	Caucasian	Positive	Negative	15.2	+/+
A335V[e]	Hemophilia	Caucasian	Positive	Negative	14.9	+/+
Y339F(1)	Hemophilia	African American	Positive	Positive	10.5	+/+
Y339F(2)	IV drug user	African American	Positive	Negative	7.3	+/+
Y339F(3)	IV drug user	African American	Positive	Negative	2.8	+/+

[a] According to 1987 CDC definition.
[b] NA = not applicable.
[c] A plus sign (+) denotes the wild-type allele.
[d] In the text, 34 Caucasians with L55Q are discussed.
[e] In the text, 12 African Americans with A335V are discussed.

ers have remained AIDS free for 7–9 years. One HIV-1–infected individual of this genotype has remained healthy for 14.9 years. Several mutations (C2OS, R223Q, 228delK, I12L, and G301V) were found exclusively in individuals who were HIV positive and AIDS negative for >11 years. In vitro analysis to determine the effect of these alterations on HIV-1 infection is in progress.

Discussion

A large number of individuals homozygous for *CCR5*-Δ*32* have now been identified, and the single naturally occurring phenotype observed in individuals with this genotype is resistance to HIV-1 infection. Since homozygosity for the *CCR5*-Δ*32* allele results in absence of CCR5 on the cell surface, absence of the CCR5 protein does not have any obvious health consequences. This notion is quite reasonable, given the large number of chemokine receptors that have similar functions and overlapping ligands (Premack and Schall 1996). The *CCR5*-Δ*32* allele appears to have originated within the past 4,000 years and may have arisen to its present frequency of 10%–15% in Caucasians because of selection against some function of the receptor (J. C. Stephens, personal communication). If the selective force that drove *CCR5*-Δ*32* to its present frequency in Caucasians persisted for a long period, then one also might expect additional mutations that alter the function of the *CCR5* gene to accumulate in the population.

A recent report by Ansari-Lari et al. (1997) has described seven *CCR5* variants, in addition to *CCR5*-Δ*32*, that have been observed in African Americans, Hispanics, Chinese, and/or Japanese but not in the 50 American Caucasians tested. Four of these variants—L55Q, R223Q, A335V, and Y339F—were also observed in our sample, and two of them (L55Q and R223Q) appear to be of Caucasian origin. Given the frequency of L55Q in our Caucasian population (.04), it is surprising that this allele was not observed in the 50 Caucasians sampled by Ansari-Lari et al. A possible explanation for the difference between the number of variants that Ansari-Lari et al. observed in Caucasians and that observed in our study could be the fact that our sample included only individuals from cohorts at high risk for HIV-1 infection, rather than a random sampling. If so, then it is likely that the variants may have an effect on HIV-1 infectivity or progression to AIDS. However, this explanation is not supported by the observation that the number of

variants found in the African American samples was similar in the two reports.

Conservation of an amino acid within a family of proteins can indicate the importance of that amino acid to a common function in that protein family; therefore, it is useful to consider the sites of amino acid variation that are deduced from the novel *CCR5* alleles. Several of the 13 nonsynonymous mutations reported herein occurred at positions that are conserved throughout members of the β-chemokine–receptor family, and three of these (C20, C101, and G301) are conserved in the α-chemokine receptor, CXCR4, as well. All of the missense mutations that occurred at conserved positions were of Caucasian origin, whereas those in African American individuals appear at amino acid sites that vary among chemokine receptors and that may more readily tolerate amino acid substitutions.

Given the striking difference between *CCR5*-Δ*32* allele frequency in Africa and that in Europe, it is not unlikely that historic selective events (e.g., infectious-disease outbreaks mediated by pathogens that utilize CCR5 as does HIV-1) may also have influenced the persistence of *CCR5* missense mutations that occur today among Africans. We cannot exclude, however, the possibility that the difference between the mutation frequencies in Africans and those in Caucasians reflects a bias in our sample of patients at risk for HIV-1.

Additional evidence suggesting a history of selective pressure targeting *CCR5* mutations is derived from the relatively high proportion of nonsynonymous, or codon-altering, mutations. Of 19 genetic variants described (table 1; also see Ansari-Lari et al. 1997) 15 (79%) were nonsynonymous. Li (1997) reported, in a comparison of 49 human genes to mouse homologues, that an average of 16% of all substitutions were nonsynonymous. An analysis of the *CCR5* gene shows that 40% of the human:mouse nucleotide substitutions are nonsynonymous. For the human:macaque *CCR5* sequence comparison, 33% of the substitutions are nonsynonymous. The high incidence (79%) of nonsynonymous substitutions among human variants is a highly significant elevation ($\chi^2 = 13.5$, $P < .0003$) of the incidence of codon-altering mutations. These results can be interpreted in at least two scenarios: it could be that (1) most *CCR5* amino acid substitutions do not affect normal CCR5 function or (2) amino acid substitutions do affect CCR5 function negatively but confer some other adaptive benefit. The latter explanation is consistent with the CCR5 role in HIV pathogenesis (Dean et al. 1996; Huang et al. 1996; Michael et al. 1997).

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