Global distribution of the *CCR2-64I/CCR5-59653T* HIV-1 disease-protective haplotype

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Objectives: Several natural polymorphisms in the genes for the human CC-chemokine receptors CCR5 and CCR2 are associated with HIV-1 disease. The *CCR2-64I* genetic variant [a G to A substitution resulting in a valine (V) to isoleucine (I) change at position 64] is in strong linkage disequilibrium with a mutation within the *CCR5* regulatory region (*CCR5-59653T*). Individuals with two CCR2-*64I* alleles are not resistant to sexual transmission of HIV-1, but progress significantly more slowly to HIV-1 disease. It is therefore important to determine the global distributions of *CCR2-64I* and *CCR5-59653T* genetic variants and define the degree of linkage between them.

Design and methods: We have developed molecular beacon-based, real-time PCR allele discrimination assays for all three chemokine receptor mutations, and used these spectral genotyping assays to genotype 3923 individuals from a globally distributed set of 53 populations.

Results: *CCR2-64I* and *CCR5-59653T* genetic variants are found in almost all populations studied: their allele frequencies are greatest (~35%) in Africa and Asia but decrease in Northern Europe. We confirm that *CCR2-64I* is in strong linkage disequilibrium with *CCR5-59653T* (96.92% of individuals had the same genotype for both CCR2-*64I* and *CCR5-59653T* polymorphisms).

Conclusions: The greater geographical distribution of the *CCR2-64I/CCR5-59653T* haplotype compared with that of *CCR5-Δ32* suggests that it is a much older mutation whose origin predates the dispersal of modern humans.

AIDS 2000, **14**:483–489

Keywords: CCR2, CCR5, polymorphisms, HIV, co-receptors

Introduction

The rate of progression of HIV-1 disease exhibits a remarkable variation among different individuals. Many host genetic factors are now known to affect disease progression rates, especially polymorphisms in genes encoding chemokine receptors [1–4]. One member of this receptor family, CCR5, is the co-receptor for the most commonly transmitted strains of HIV-1 [5–9]. A deletion of 32 nucleotides in the *CCR5* gene (denoted *CCR5-Δ32*) results in a truncated protein that is not expressed on the cell surface; individuals homozygous for this deletion have an absolute resistance to infection by CCR5-using (R5) HIV-1 variants [10–14], although infection by CXCR4-using (X4) strains can occur infrequently [15–17]. Heterozygotes for *CCR5-Δ32* do not resist HIV-1 infection but progress more slowly (by approximately 2 years) to AIDS [10, 11,14,18–20]. Another mutation in a closely linked chemokine receptor gene, *CCR2*, also affects HIV-1

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Sponsorship: Supported by the National Institutes of Health under RO1 AI43868 (L.G.K.), AI41420 (J.P.M.), the Wellcome trust (J.J.M.) and the Pediatric AIDS Foundation, of which J.P.M. is an Elizabeth Glaser Scientist.

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Received: 8 December 1999; accepted: 22 December 1999.

disease progression. This mutation, denoted CCR2-*64I*, is a G to A substitution that results in a replacement of valine with isoleucine at position 64 of the CCR2 protein [21]. The homozygous *CCR2-64I/64I* phenotype has no effect on HIV-1 infection, but is associated with a delayed progression to disease. Heterozygotes for *CCR2-64I* do not progress more slowly to AIDS [21,22]. As the valine to isoleucine substitution is a conservative change in a protein that is rarely used as a HIV-1 co-receptor, this was an unexpected finding; indeed, no effects of the 64I substitution on CCR2 function have been found [23]. Further analysis of the *CCR5-CCR2* gene family showed that *CCR2-64I* was in strong linkage with a C to T mutation approximately 12 kb downstream in the regulatory region of the *CCR5* gene (*CCR5-59653T*). This suggested that the *CCR5-59653T* mutation (or another unrecognized one with a similar degree of linkage) may be the one that affects HIV-1 disease progression, although this is not yet proven [22]. The precise degree of linkage between *CCR2-64I* and *CCR5-59653T* has also not yet been established: initially, the association between *CCR2-64I* and *CCR5-59653T* was thought to be absolute [22], but exceptions have recently been reported [24]. We address this issue here.

Methods

The samples studied include many of those in which we previously determined the distribution of *CCR5-Δ32* [25–28] and a series of further populations obtained as part of epidemiological surveys.

To genotype the three polymorphisms (*CCR5-Δ32, CCR2-64I* and *CCR5-59653T)* in each sample from all 54 populations, we used spectral genotyping [29]. The spectral genotyping assays for *CCR5-Δ32* and *CCR2-64I* have been described previously [22,29]. To genotype the *CCR5-59653C/T* polymorphism, we designed a new assay based on the general operational principle described previously [29]. One molecular beacon, labeled with 6-carboxyfluorescein (FAM), recognizes the wild-type *CCR5-59653C* allele (position 59653 of GenBank file U95626) the other, labeled with hexachlorofluorescein (HEX), is for the *CCR5-59653T* genetic variant (C at position 59653). Their nucleotide sequences were respectively: FAM-5′-CCGCGGTTTGCCAAATGTCTTCTATCGCGG-3′-DABCYL and HEX-5′-CCGCGGTTTGCAAATATCTTCTATCGCGG-3′-DABCYL, where DABCYL is the quencher 4-(4′-dimethylaminophenylazo) benzoic acid and underlined sequences indicate the complementary sequences forming the hairpin structure. The primers were 5′-CAGAAGAGCTGAGACATCCGT-3′ and 5′-CATTCCAAACTGTGACCCTTTCC-3′. The real-time PCR conditions and data analysis methods have been described previously [22,29]. Each 50 μl PCR contained 0.5–5.0 μg of genomic DNA, 0.25 μM of each molecular beacon, 0.5 μM of each primer, 0.25 mM dATP, 0.25 mM dCTP, 0.25 mM dGTP, 0.25 mM dTTP, 2.5 U AmpliTaq Gold DNA polymerase (PE Biosystems, Foster City, California, USA), 50 mM KCl, 3.5 mM $MgCl_2$, and 10 mM Tris–HCl (pH 8.3). Forty-five cycles of amplification (denaturation, 94°C for 15 s; annealing, 55°C for 30 s; polymerization 72°C for 30 s) were performed in a spectrofluorometric thermal cycler (ABI 7700; PE Biosystems). Those samples that had previously been typed for *CCR5-Δ32* by methods involving PCR and agarose gel electrophoresis all yielded identical genotypes when the spectral genotyping assays were used. The high sensitivity of the spectral genotyping method allowed for some individuals to be genotyped who could not be typed by the original procedure.

Results

The geographical distribution of *CCR5-Δ32* is now well characterized. It is present at high frequencies (~12–15%) in populations of northern European origin, and decreases in frequency in a southeast cline towards the Mediterranean [25–28]. Outside Europe and Northern America, it is seen at low frequencies (2–5%) in the Near East and India, and is absent elsewhere apart from isolated occurrences that are probably the result of recent European gene flow (i.e., into North America). The restricted distribution of *CCR5-Δ32* indicates that the mutation arose relatively recently in human history [30,31]. The distributions of CCR2-*64I* and *CCR5-59653T* have been less thoroughly studied, although these alleles are more common in African–American and Hispanic populations than they are in Caucasian–Americans, in contrast with *CCR5-Δ32* [21,22,24]. Here we examine in detail the frequencies of *CCR2-64I*, *CCR5-59653T* and their combined haplotype in a globally distributed set of populations, including many that we have previously typed for *CCR5-Δ32*. We also include data on the distribution of *CCR5-Δ32* in an additional set of populations.

Table 1 shows the distribution of *CCR5-Δ32*, *CCR2-64I* and *CCR5-59653T* worldwide. Individuals carrying the *CCR2-64I* and *CCR5-59653T* alleles can be seen in most of the world's populations. The contrast between the distribution of *CCR5-Δ32* and that of *CCR2-64I* and *CCR5-59653T* is striking. The Europe-centered distribution of *CCR5-Δ32* reported previously is upheld by the new data presented here, and it differs greatly from the globally ubiquitous presence of *CCR2-64I* and *CCR5-59653T* (Fig. 1). The fre-

Table 1. Global distribution of CCR5 and CCR2 chemokine receptor polymorphisms.

		CCR5-Δ32						*CCR2-64I*						*CCR5-59653T*						
Population	n	NA	wt/ wt	wt/ mut	mut/ mut	Frequency (%)	HWE	NA	wt/ wt	wt/ mut	mut/ mut	Frequency (%)	HWE	NA	wt/ wt	wt/ mut	mut/ mut	Frequency (%)	HWE	Congruence (%)
Europe	1198	12	1050	129	7	6	0.17	13	900	257	28	13.2	0.06	98	831	244	25	13.4	0.16	98.1
Caucasian American	146		133	13		4.5		3	104	33	6	15.7		77	49	17	3	16.7		97.1
Caucasian American	549	2	455	87	5	8.9		1	440	103	5	10.3		2	432	109	6	11.1		98.2
Britain[a]	47	2	36	7	2	12.2		2	39	5	1	7.8		3	38	5	1	8.0		100.0
Romania	11		10	1		4.5			8	3		13.6			8	3		13.6		100.0
Cyprus	310	7	287	16		2.6		1	213	87	9	17.0		5	210	85	10	17.2		96.7
Greece	135	1	129	5		1.9		6	96	26	7	15.5		11	94	25	5	14.1		96.8
Middle East	421	8	354	54	5	8	0.08	16	324	75	6	10.7	0.49	20	322	76	3	10.2	0.52	97.2
Israel, Ashkenazim	147		108	36	3	14.3		5	117	24	1	9.2		3	117	26	1	9.7		95.7
Israel, Sephardim	66		61	4	1	4.5		4	45	16	1	14.5		1	49	16		12.3		93.4
Yemen	96	6	89		1	1.1		5	69	20	2	13.2		5	71	19	1	11.5		96.7
Mekhelta highlands[a]	64		56	8		6.3		2	54	7	1	7.3		3	53	7	1	7.4		100.0
Mekhelta lowlands[a]	48	2	40	6		6.5			39	8	1	10.4		8	32	8		10.0		100.0
Asia	740	5	717	16	2	1.4	0.00	17	552	153	18	13.1	0.06	23	528	169	20	14.6	0.15	95.8
Russia[a]	47	1	38	7	1	9.8		3	44			0.0		1	40	6		6.5		93.0
Mongolia[a]	58		58			0.0		5	24	22	7	34.0		3	23	25	7	35.5		92.3
Pakistan[a]	36	2	32	2		2.9			34	2		2.8			34	2		2.8		100.0
Punjab[a]	34		33	1		1.5			31	3		4.4			30	4		5.9		97.1
Gujerat[a]	31		29	1	1	4.8		1	26	4		6.7			22	9		14.5		86.7
Sind[a]	28		27	1		1.8		1	20	6	1	14.8			22	6		10.7		92.6
Thailand[a]	60		60			0.0			42	15	3	17.5			42	15	3	17.5		100.0
Philippines[a]	66		66			0.0			59	7		5.3			60	6		4.5		98.5
Hong Kong[a]	50		50			0.0		1	35	11	3	17.3		1	36	10	3	16.3		98.0
Kota Kinabalu, Malaysia[a]	179	1	178			0.0			132	44	3	14.0			126	49	4	15.9		95.0
Taiwan Ami[a]	23		23			0.0		2	13	8		19.0		1	15	7		15.9		95.2
Taiwan Atayal[a]	20		20			0.0		3	13	4		11.8		1	14	5		13.2		100.0
Taiwan Bunun[a]	20	1	19			0.0			16	4		10.0			16	4		10.0		100.0
Taiwan Paiwan[a]	21		21			0.0			17	4		9.5			17	4		9.5		100.0
Asian American	67		63	4		3.0		1	46	19	1	15.9		16	31	17	3	22.5		88.2

continued overleaf

Table 1. (*continued*)

Population	n	*CCR5-Δ32*						*CCR2-64I*						*CCR5-59653T*						Congruence (%)
		NA	wt/wt	wt/mut	mut/mut	Frequency (%)	HWE	NA	wt/wt	wt/mut	mut/mut	Frequency (%)	HWE	NA	wt/wt	wt/mut	mut/mut	Frequency (%)	HWE	
Africa	573	1	562	10	0	0.9	0.83	11	391	149	22	17.2	0.11	225	239	95	14	17.7	0.25	96.5
Gambia	47		47			0.0			43	4		4.3			41	5	1	7.4		95.7
Central African Republic[a]	52		52			0.0			33	17	2	20.2			30	20	2	23.1		94.2
Uganda	6		6			0.0			4	2		16.7			4	2		16.7		100.0
Kenya	7		7			0.0			2	5		35.7			2	5		35.7		100.0
Tanzania	7		7			0.0			5	1	1	21.4			5	1	1	21.4		100.0
Malawi	13		13			0.0		1	8	4		16.7		1	8	4		16.7		100.0
Madagascar Betsileo	42		42			0.0		2	29	9	2	16.3		5	27	8	2	16.2		97.3
Madagascar Bezanozano	16		16			0.0			7	9		28.1			7	9		28.1		87.5
Madagascar Merina	42		42			0.0		3	31	5	3	14.1		3	28	8	3	17.9		91.9
Madagascar Sihanaka	19		19			0.0		1	8	7	3	36.1		1	8	7	3	36.1		100.0
African American	322	1	311	10		1.6		4	221	86	11	17.0		215	79	26	2	14.0		94.3
Oceania	550	12	537	1	0	0.1	0.98	15	463	68	4	7.1	0.39	12	460	74	4	7.6	0.59	99.0
Papua New Guinea[a]	96	4	92			0.0		5	60	29	2	18.1		3	61	29	3	18.8		100.0
Banks and Torres Islands	38		38			0.0			33	5		6.6			33	5		6.6		100.0
Vanuatu, Maewo	23	1	22			0.0		1	16	5	1	15.9		1	16	5	1	15.9		100.0
Vanuatu, Santo	18		18			0.0			11	7		19.4			11	7		19.4		100.0
Nauru	30		30			0.0		1	29			0.0			29	1		1.7		100.0
Majuro	29		29			0.0			28	1		1.7			28	1		1.7		100.0
Truk	30		30			0.0			27	3		5.0			27	3		5.0		100.0
Guam[a]	30		30			0.0		1	26	3		5.2		1	26	3		5.2		100.0
Palau	30		30			0.0			26	4		6.7			25	5		8.3		96.7
Kiribati	30		30			0.0			30			0.0			30			0.0		100.0
Pohnpei	30	1	28	1		1.7		1	25	4		6.9		1	25	4		6.9		100.0
Kapingamarangi	30		30			0.0			29		1	3.3			27	3		5.0		90.0
French Polynesia[a]	96	2	94			0.0		2	87	7		3.7		2	86	8		4.3		98.9
Tonga	40	4	36			0.0		4	36			0.0		4	36			0.0		100.0
Americas	441		421	20	0	2.3	0.63	8	326	94	13	13.9	0.06	171	205	52	13	14.4	0.00	97.8
Haiti	67		67			0.0		3	48	13	3	14.8		3	48	13	3	14.8		100.0
Hispanic American	374		354	20		2.7		5	278	81	10	13.7		168	157	39	10	14.3		95.6
Overall	3923																			97.2

[a]CCR5-*Δ*32 data previously reported in Martinson *et al*. NA, Not determined due to depletion of DNA samples; HWE, Hardy–Weinberg equlibrium comparisons; value shown are the exact probabilities of obtaining the observed distribution under HWE.

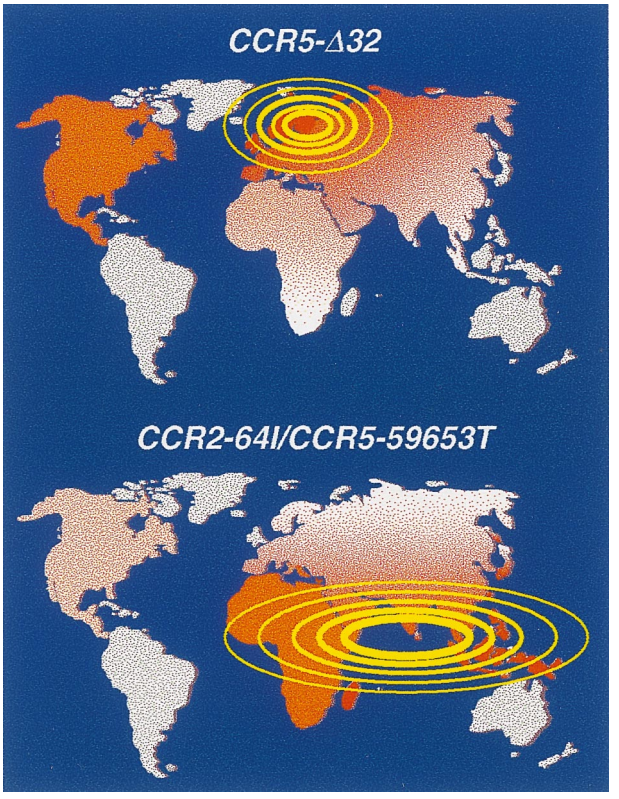

Fig. 1. Global schematic distribution of the *CCR5-Δ32* (upper) and *CCR2-64I/CCR5-59653T* (lower) allele frequencies based on the results in Table 1. The white-to-red color gradient represents increasing allele frequencies. The ellipsoids indicate the approximate boundaries of the populations with the highest allele frequencies.

quencies of these genetic variants are highest (> 35%) in sub-Saharan African populations, but they are also observed in Asian populations. Slightly lower frequencies are present in Europeans and Caucasian–Americans, and the lowest are found in Pacific Islander populations.

Another striking feature of the distribution of *CCR2-64I* and *CCR5-59653T* is the close association between the allele frequencies of these two polymorphisms. Table 1 shows the congruence between the genotypes for these alleles; i.e., the number of times that an individual was either heterozygous or homozygous for both *CCR2-64I* and *CCR5-59653T*. Individuals who were only informative for one of the genotypes were excluded from the congruence calculations. In many populations the association is absolute, indicating a strong degree of linkage between the two polymorphisms. A total of 3341 individuals were genotyped for both polymorphisms: where the congruence was not absolute, in 33 cases an individual had a *CCR2-64I* allele but not a *CCR5-59653T* one (0.99%), and in 70 cases an individual had a *CCR5-59653T* allele but lacked *CCR2-64I* (2.09%). These observations are consistent with the genealogy proposed by Mummidi *et al.* in which the *CCR5-59653T* polymorphism arose first, and a later mutation on a *CCR5-59653T* chromosome gave rise to the *CCR2-64I* allele [24]. The slightly greater prevalence of the *CCR5-59653T* chromosome reflects its ancestral status, while the low frequency of chromosomes bearing solely *CCR2-64I* is consistent with it being produced from a *CCR2-64I/CCR5-59653T* chromosome by recombination with a normal chromosome. Such a recombination event would also produce a chromosome carrying *CCR5-59653T* alone, which would account for some of the occurrences of this chromosome. Calculation of congruence between *CCR5-Δ32* and either *CCR2*-64I or *CCR5-59653T* showed no instances of crossing-over between these polymorphisms.

The degree to which the genotype frequencies at each locus agree with Hardy–Weinberg equilibrium (HWE) expectations is also shown in Table 1. This was determined at the regional level, and not for individual populations, as many of the individual samples were too small to allow the agreement with HWE to be calculated accurately. Agreement with HWE ($P > 0.05$) was seen in 16 out of 18 comparisons. In some cases, the expected values obtained for some genotype frequencies were so low as to introduce bias into the chi-square calculation [32], which may explain the disagreement seen at *CCR5-Δ32* in Asian populations. The other exception (*CCR5-59653T* in the Americas) was due to an observed excess of homozygotes and a deficit of heterozygotes. The reason for this is not clear, but instead of reflecting an aspect of population structure, this could be a Type I error; a probability threshold of 0.05 would result in HWE being falsely rejected in one out of every 20 tests performed. We conclude that there is little or no selective or other effect of *CCR2-64I* or *CCR5-59653T* that is sufficient to perturb the genotype frequencies.

Discussion

The global distribution of both *CCR5-59653T* and *CCR2-64I* indicates that these mutations are much older than *CCR5-Δ32*. The restricted distribution of *CCR5-Δ32* has lead to the suggestion that it arose very recently; age estimates based on flanking STR haplotype analysis have ranged from 750 to 2500 years, although a precise date has yet to be generally accepted [30,31]. Certainly the *CCR5-59653T* and *CCR2-64I* mutations are much older than this. Their presence in virtually all of the world's populations would imply that these mutations arose prior to the dispersal of

modern *Homo sapiens* from an African ancestral population more than 100 000 years ago. As human populations have only become exposed to HIV-1 within the past two or three generations at most, any resistance to HIV-1 disease progression conferred by any of these mutations cannot explain their persistence at polymorphic frequencies. It is possible for polymorphisms that have no selective advantage or disadvantage to persist for long periods of time, in which case their frequencies fluctuate randomly due to the stochastic processes of genetic drift. Under these circumstances, the allele frequencies would be expected to fluctuate between populations over a great range, from total absence in some populations to fixation in others. The majority of allele frequencies for *CCR5-59653T* and *CCR2-64I* are within a narrow range (10–30%); the persistence of this frequency in such a wide range of populations suggests that random genetic drift is not responsible for the pattern seen. The only exceptions to this are seen in the populations of Oceania, but these populations are known to have undergone a dramatic reduction in size in the recent past that has drastically affected their genetic composition in other ways [33].

It may be that one or other of the *CCR5-59653T* and *CCR2-64I* polymorphisms confers a selective advantage against other, unknown infectious agents. The precise nature of the interactions between chemokines, their receptors and the other components of the chemotactic response to infection is not well understood. The ability of the mutations described here to delay the progression of HIV-1 infection and disease implies a potential for the conferral of a selective advantage against other infectious organisms that activate these components of the immune response. As more chemokine receptor variants become identified and their roles characterized in detail, then our understanding of both the mechanism of HIV-1 infection and the long-term effects of natural selection in human populations will continue to improve.

Acknowledgements

The authors thank the many researchers who provided the samples for our original study of CCR5-Δ32, and to C. Christodoulou and A. Hatzakis for additional samples.

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